FINAL REPORT

W-113-R(SI)

Avian Cholera in Canada Geese on Southern Illinois Wintering Areas

Submitted by:

Cooperative Wildlife Research Laboratory, SIUC

Presented to:

Illinois Department of Conservation

August 31, 1994

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FINAL REPORT

State of Illinois

W-113-R(SI)

Project Period: 1 July 1991 through 30 June 1994

Study: Avian Cholera in Canada Geese on Southern Illinois Wintering Areas

Prepared by Robert J. Gates and Alan Woolf
Cooperative Wildlife Research Laboratory
Southern Illinois University at Carbondale

Need: Avian cholera, caused by the bacterium Pasteurella multocida has become an important infectious disease in populations of Canada geese (Branta canadensis) and other waterfowl that winter on refuges in southern Illinois. Sporadic outbreaks of the disease have been documented at Union County Conservation Area (CA) since 1979. Epizootics of avian cholera have occurred with increasing frequency, claiming up to several thousand geese at Union County and Horseshoe Lake Conservation Areas (CA) in recent years (1986 - 1990). Disease outbreaks have tended to occur at the same locations within Union County and Horseshoe Lake CA, and under similar environmental conditions each year. The increasing frequency and recurrent pattern of cholera outbreaks suggest that the disease has become enzootic on portions of southern Illinois waterfowl refuges. With recent growth that has occurred in the Mississippi Valley Population (MVP) of Canada geese that winters in southern Illinois, there has been greater concern over the potential for large disease outbreaks to claim substantial proportions of winter refuge populations at Union County and Horseshoe Lake CA.

The epizootiology of avian cholera must be understood before management recommendations can be formulated to prevent disease outbreaks or measures can be implemented measures that would limit disease transmission and mortality. Comparisons of areas where the disease appears endemic with areas where outbreaks do not normally occur could identify physical and biological factors that predispose the recurrence of disease on a particular site. Physical manipulations of habitat (water levels, vegetation composition, etc.) could then be performed to eliminate or minimize the spread of disease from areas where it may be endemic. Understanding of the role (if any) of physiological condition and environmental stressors in disease and other natural mortality will enable managers to anticipate and cope with conditions that cause infectious disease.

Objectives:

1. Monitor and compile information on the onset, duration, and distribution of avian cholera outbreaks among Canada geese wintering in and around Union County and Horseshoe Lake Conservation Areas.

2. Determine environmental, physical, and biological factors associated with avian cholera outbreaks, and characteristics of specific areas where the disease appears to be endemic.

3. Evaluate relationships of physiological condition and environmental stresses to the occurrence of disease outbreaks and other natural sources of natural mortality.

EXECUTIVE SUMMARY

We began this project with the goal of understanding the epizootiology of avian cholera to a degree that we could provide managers a means to predict, or even reduce risk of epizootics on southern Illinois refuges. Although the etiologic agent of avian cholera is well known, important issues in the epidemiology of the disease remain useful and valid research topics.

We planned the study around the assumption that avian cholera had established itself as an enzootic disease with an annual occurrence at southern Illinois waterfowl refuges. Our approach included 4 jobs designed to address 3 objectives. Job 1 used the strategy of descriptive epidemiology to fully characterize the disease. Job 2 was designed to analyze the spatial and temporal patterns in order to define determinants of outbreaks (e.g. any agent, host, or environmental factor which directly or indirectly increases frequency of occurrence of the disease). Job 3 was planned to determine what host conditions might predispose the wintering populations to outbreaks. Job 4 was planned to summarize the data and published literature to achieve the overall project goal.

Avian cholera did not occur as a recognizable outbreak during the study period. In the first year of the study, we rejected our original hypothesis that avian cholera was enzootic at southern Illinois refuges. Unable to compare enzootic sites in contrast to those nearby where the disease did not occur, we altered the focus and emphasis of Job 2 and instead sought information concerning the source and reservoir mechanism of the causative agent. In collaboration with staff of the National Wildlife Health Research Center (NWHRC), we tested the ability and efficiency of selective culture media to detect Pasteurella multocida in environmental samples, especially water.

The lack of cholera (or any other disease) outbreaks also forced us to change the approach to conduct of Job 3. Since we could not compare physiological condition and stress levels in clinically normal, morbid, and moribund birds, we instead developed baseline data on tissue levels of various hormonal indicators of stress and condition. These data should prove useful in future outbreak investigations.

Although the failure of avian cholera to reappear during the course of this study forced us to alter the research design, project goals and objectives were met. Improved diagnostic capability (e.g. the selective media) was a product of this study, as was a clearer description of the temporal and spatial aspects of avian cholera outbreaks in the Mississippi Flyway.

INTRODUCTION

Avian cholera, a bacterial disease caused by Pasteurella multocida, has a recent history of causing substantial mortality in Canada geese (Branta canadensis) that winter in southern Illinois. Epornitics have occurred southern Illinois wintering areas since 1979. Disease outbreaks have tended to occur at the same locations within Union County and Horseshoe Lake CA, suggesting presence of enzootic foci.

Despite much effort in monitoring and studying disease outbreaks as they occur, the epizootiology of avian cholera is not fully understood. Two primary hypotheses have been proposed to explain the occurrence of disease outbreaks (Brand 1984, Botzler 1991, Wobeser 1992). The environmental reservoir hypothesis predicts that P. multocida persists in water, soil, or reservoir species in certain areas as enzootic foci that are sources of infection for susceptible waterfowl. The carrier bird hypothesis predicts that P. multocida persists latently in healthy waterfowl that shed virulent organisms under conditions of nutritional, energetic, or environmental stress, eventually leading to die-offs. A secondary hypothesis, not mutually exclusive with the 2 above is that nutritional deficiencies and exposure to mycotoxins (Smith et al. 1990) and contaminants predispose birds to disease by reducing resistance to infection with P. multocida (Wobeser 1992). More complete understanding of the epizootiology of avian cholera is necessary to implement control measures that would limit disease transmission and risk of outbreak. This study was initiated in July 1991 to explore facets of the above hypotheses and to determine the physical, ecological, and biological factors associated with avian cholera outbreaks on and near Union County CA and Horseshoe Lake CA.

ACKNOWLEDGEMENTS

We acknowledge the contributions of numerous participants in this study. L. Cicnjak-Chubbs, M. J. O'Leary, P. A. Shelton, J. P. Shimp, and P. J. Bergmann (SIUC Cooperative Wildlife Research Laboratory) assisted in collection of field data, and water and goose tissue samples. R. E. Connors, (SIUC Dept. of Microbiology), L. Cicnjak-Chubbs, and M. K. Moore (National Wildlife Health Research Center) prepared media and performed microbiological analyses. A. G. Amador (SIUC School of Medicine) performed and analyzed the results of radioimmunological assays. M. Friend, C. J. Brand, and J. I. Price graciously provided cooperation and access to facilities of the National Wildlife Health Research Center (NWHRC). R. M. Windingstad (NWHRC) provided valuable advice and access to unpublished data. E. K. Smith and L. I. Tripp (SIUC Vivarium) provided laboratory space and other assistance. R. J. Kawula, P. A. Shelton, and J. P. Shimp prepared field maps and figures. We also thank D. D. Thornburg, R. M. Whitton, and D. A. Woolard (Illinois Dept. of Conservation) for logistical and administrative support, and access to unpublished data.

STUDY AREAS

Field work was conducted at Union County and Horseshoe Lake CAs located in Union and Alexander counties, in southern Illinois. Both areas were managed primarily to provide resting and feeding habitat for wintering and migrating populations of Canada geese. Union County CA included a 1,320 ha refuge area that was closed to hunting and a 830 ha public hunting area that was open to waterfowl hunting during the legal season on a permit basis. The refuge portion of Union County CA refuge included 590 ha of croplands that were annually planted to corn, soybeans, winter wheat, other forage crops, or left fallow (Moritz 1992). Additional crops (primarily corn and winter wheat) were planted on the public hunting area, as well as surrounding private lands. Open roost water was maintained during dry and/or cold periods by pumping groundwater with 4 pumps located on lakes and wetlands used by geese. Union County wintered peak populations of 68,000 - 104,000 Canada geese during the winters 1991-92, 1992-93 and 1993-94.

Horseshoe Lake CA included a 1,762 ha refuge and a 1,936 ha area open to hunting that were managed similarly to Union County CA (Moritz 1992, Brandon et al. 1994). Crops were planted on about 760 ha of the Horseshoe Lake CA refuge, and open water was maintained by pumping ground water on one small wetland area located in the center of the refuge. Horseshoe Lake Ca wintered peak populations of 130,000 - 205,000 Canada geese during the winters 1991-92, 1992-93 and 1993-94.

Microbiological work and data analyses were performed at the Cooperative Wildlife Research Laboratory, Southern Illinois University at Carbondale (SIUC), and in laboratories of the SIUC Department of Microbiology and SIUC Vivarium. Radioimmunoassays of selected organ tissues were performed at the SIUC School of Medicine in Springfield, IL.

Job 1: Characteristics of Avian Cholera Outbreaks

Objective: Monitor and compile information on the onset, duration, and distribution of avian cholera outbreaks among Canada geese and other waterfowl wintering in and around Union County and Horseshoe Lake Conservation Areas.

INTRODUCTION

Numerous sources have described previous epornitics of avian cholera in wild birds (Brand 1984; Windingstad et al. 1984, 1988; reviewed by Botzler 1991) or reported environmental features associated with outbreaks of the disease in North America (Gordon 1989, Smith et al. 1989, 1990, Smith and Higgins 1990; Windingstad et al. In Press). Characteristic features of most documented outbreaks of the disease are that they typically occur under adverse weather conditions and crowding of waterfowl on limited areas of open water during winter and/or spring migration (Wobeser 1992). Geographical patterns of disease outbreaks and the distribution of enzootic sites suggest that the disease is spread by contact between different species or populations when they become mixed on winter areas or migration routes (Friend 1987). Brand (1984) suggested that snow geese (Chen caerulescens) may be important carriers of the disease, introducing avian cholera to new areas or populations. There are indications that avian cholera may be spread within the Mississippi

Flyway by contact between Canada geese of the Mississippi Valley (MVP) and Eastern Prairie (EPP) Populations (Brand 1984, Windingstad et al. In Press).

We summarized the histories of avian cholera epornitics in the Mississippi Flyway and in southern Illinois to determine broad spatial and temporal patterns of disease outbreaks. This work was conducted primarily to facilitate planning and execution of Jobs 2 and 3 below. Routine field monitoring also was conducted to detect the onset, duration, and severity of disease outbreaks at Horseshoe Lake and Union County CA during the years of this study. Intensive monitoring was conducted at Union County CA during all 3 years of the project, and at Horseshoe Lake CA only during 1991-92. After summarizing the histories of disease outbreaks at these 2 refuges, it was apparent that outbreaks of avian cholera at Horseshoe Lake CA occurred after appearance of disease at Union County CA (see below). Consequently, field monitoring efforts were reduced at Horseshoe Lake CA in 1992-93 and were discontinued in 1993-94. This allowed monitoring efforts to be intensified at Union County CA in the last 2 years of the study.

METHODS

Outbreak Histories

We reviewed published and unpublished information on the timing and location of avian cholera outbreaks that caused documented mortality within the Mississippi Flyway, and more specifically, within the range of MVP Canada geese. Published and unpublished reports of avian cholera epornitics were summarized to document geographic and temporal patterns of disease outbreaks after avian cholera was first recorded in the Mississippi Flyway in 1979. Disease outbreaks reported to the NWHRC and appearing in quarterly Supplements to the Journal of Wildlife Diseases also were summarized. Data on the timing and duration of mortality due to avian cholera in Canada geese of the Mississippi Valley Population (MVP) were compiled from published literature, unpublished Illinois Department of Conservation (IDOC) reports, and unpublished data (SIUC Cooperative Wildlife Research Laboratory).

Outbreak Monitoring

Intensive monitoring to detect onset, duration, and mortality associated with disease outbreaks was conducted during the 3 years of this study. Routine surveys were conducted 26 times on Horseshoe Lake CA and Union County CA between 19 December 1991 and 28 February 1992. One survey was conducted every 2-3 days on each area. Surveys began in the morning after 0700 hours. Nine wetland and lake sites at Horseshoe Lake CA, and 18 sites at Union County CA were monitored (Figs. 1 and 2) in 1991-92 based on prior knowledge of areas where geese concentrated during winter, and where dead and moribund geese were recovered during previous outbreaks. During 1992-93, numbers of geese using enzootic and other sites were recorded concurrently with monthly collection of water samples for isolation of P. multocida (see Job 2, Environmental and Biological Factors) during October 1992 through February 1993. More intensive monitoring was conducted at Union County CA between 19 December 1992, and 12 February 1993 when 19 wetland and lake sites were visited a total of 18 times. Monitoring efforts were further intensified at Union County CA in 1993-94 when a vehicle survey route was established that provided visual

Figure 1. Wetland and lake sites within Union County Conservation Area in southern Illinois that were monitored to detect onset of avian cholera, and where water samples were collected during winters 1991-92 and 1992-93.

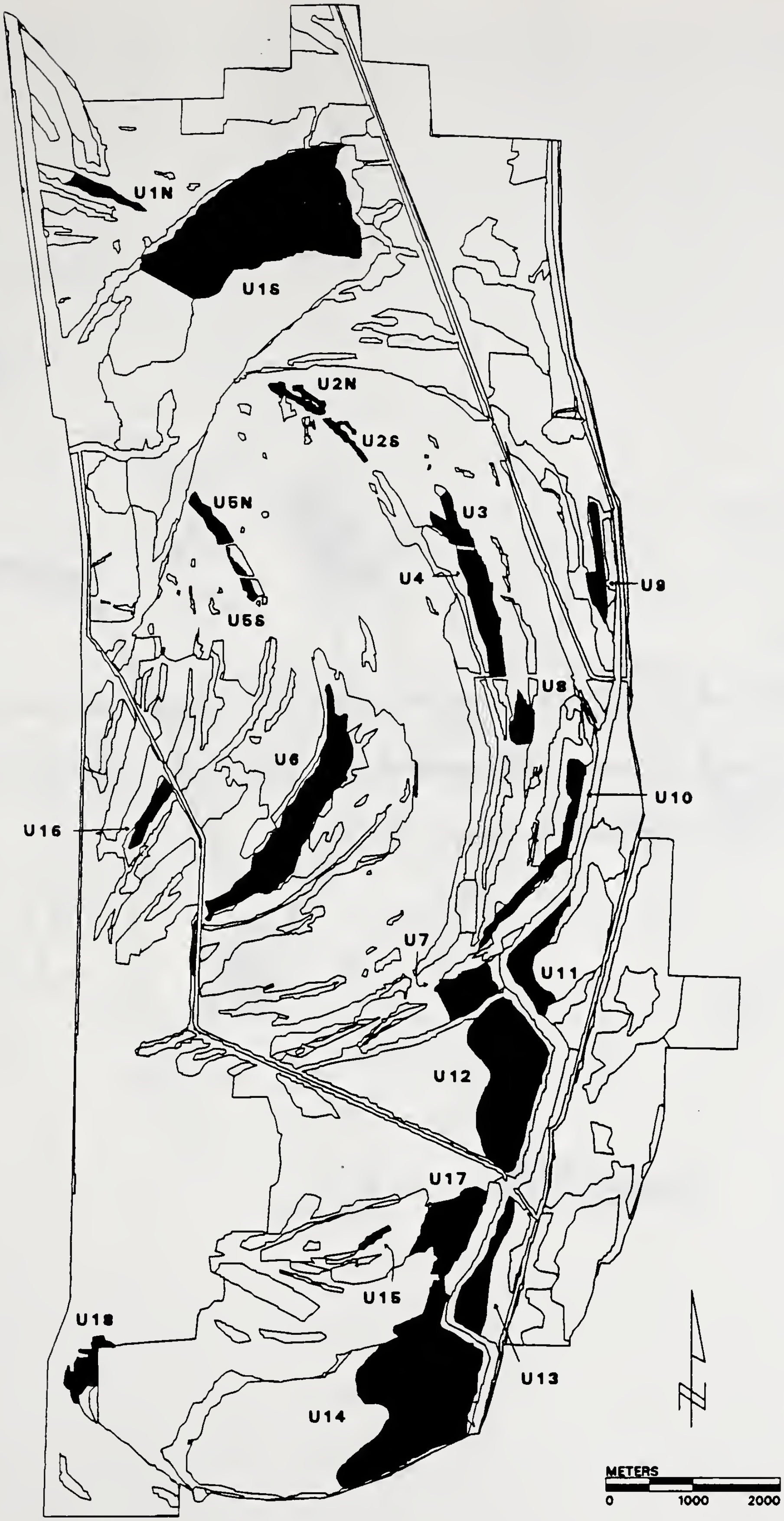
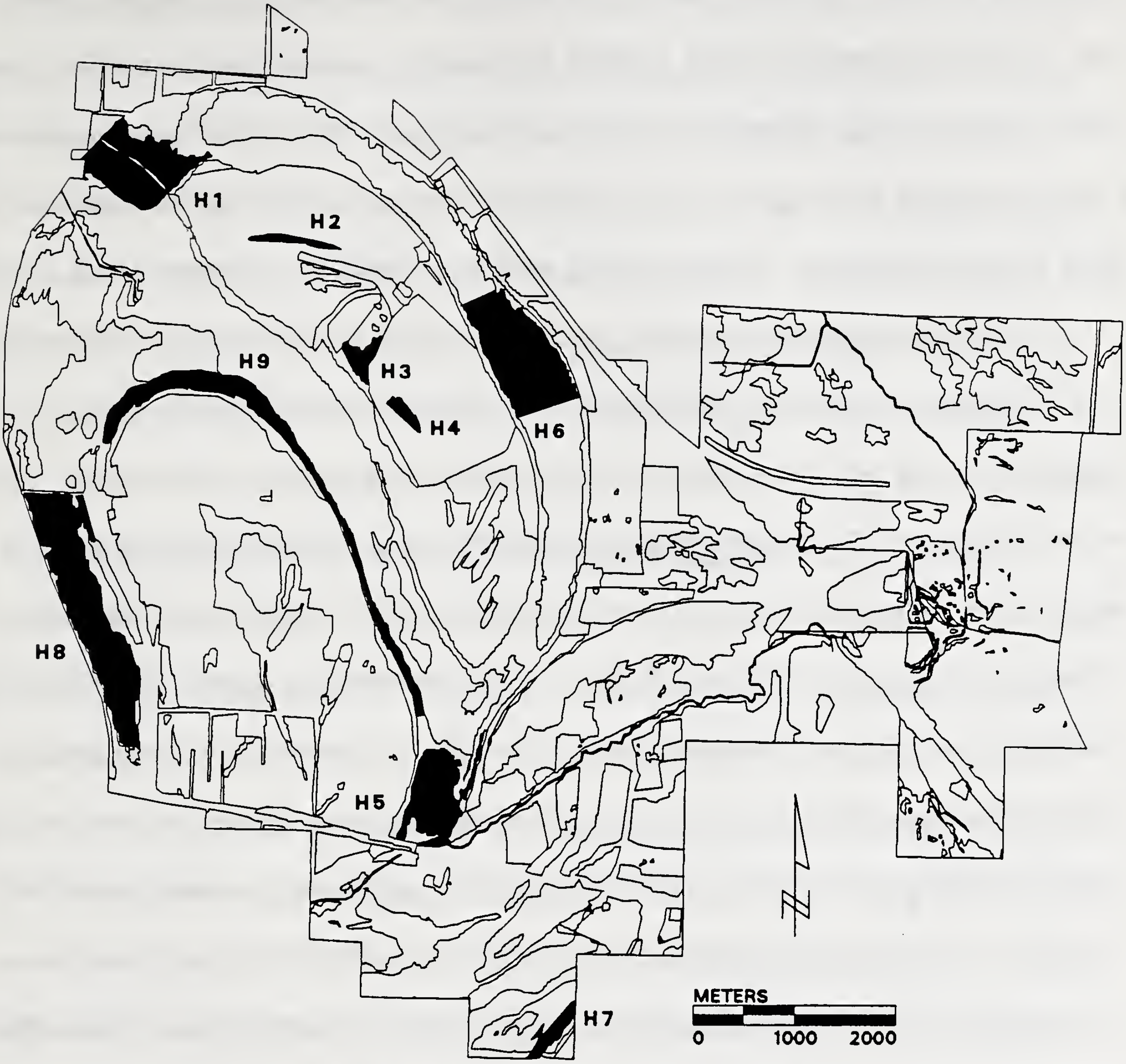

U1N
U1S
U2N
U2S
U5N
U3
U4
U9
U5S
U8
U6
U10
U16
U7
U11
U12
U17
U15
U18
U13
U14
N
METERS
0
1000
2000

Figure 2. Wetland and lake sites within Horseshoe Lake Conservation Area in southern Illinois that were monitored to detect onset of avian cholera, and where water samples were collected during winters 1991-92 through 1993-94.


H1
H2
H3
H4
H5
H6
H7
H8
H9
METERS
0
1000
2000
N

access to 97 wetlands including shallow emergent wetlands, ponds, and lakes throughout Union County CA. All wetland sites where previous disease outbreaks occurred were included on the route. Numbers, species, and behavior of waterfowl using wetlands visible from the route were recorded during 3 diurnal time periods: morning (sunrise to 1000), midday (1000 to 1400), and evening (1400 to sunset) each day that the route was driven. Routine monitoring was initiated on 14 October 1993 and continued through the peak of spring waterfowl migration. Wetland surveys were terminated after 6 February 1994.

Waterfowl observed using wetland and lake sites were counted to obtain information on bird densities. Areas of each wetland and lake site monitored were determined by measuring digitized National Wetlands Inventory (NWI) polygons using a geographic information system. Non-forested shallow wetlands were differentiated from lakes and deeper impoundments on the basis of their classification by NWI (palustrine vs. lacustrine systems; Cowardin et al. 1979), size, depth, and water permanency. Mean densities of all waterfowl at each site were calculated from numbers of birds observed during each visit. These densities reflected diurnal use of wetland and lake sites since observations were not recorded before birds left roosting areas in the morning, or after birds returned to roosts in the evening. Presence of dead and moribund waterfowl was recorded during all years at each location, and any intact carcasses that could be retrieved were recovered. Wetland sites and surrounding areas were scanned or searched on foot for dead and moribund birds. Intact carcasses and moribund waterfowl were collected for later necropsy to detect gross lesions indicating infectious diseases or trauma.

RESULTS AND DISCUSSION

Outbreak Histories

Mississippi Flyway.--Union County and Horseshoe Lake CAs were the first sites where waterfowl mortality due to avian cholera was documented in the Mississippi Flyway (Windingstad et al. 1983). The disease was detected during weekly surveillance transects that were conducted at Union County CA, Horseshoe Lake CA, and Crab Orchard National Wildlife Refuge (NWR). Outbreaks of avian cholera that occurred in southern Illinois during 1978-79 (Table 1) preceded widespread disease outbreaks in the Mississippi Flyway during the following year (Brand 1984). Following the 1978-79 outbreak in southern Illinois, cholera outbreaks were also reported in Canada geese of the Eastern Prairie Population (EPP) in Missouri and Minnesota, and in snow geese (Chen caerulescens) in the Northwest Territories of Canada (Table 1). Brand (1984) believed that the disease outbreaks of 1979-80 were initiated by infected geese that survived previous epornitics in snow geese nesting in western Hudson Bay, and/or in MVP geese exposed to the 1978-79 outbreaks in southern Illinois. Similarly, disease outbreaks that were widespread within the Mississippi Flyway in 1989-90 were also preceded by consistent outbreaks in southern Illinois during the preceding 3 years. The winter of 1989-90 had the largest number of recorded outbreaks, with mortality reported on 13 of 21 areas where avian cholera was reported during 1978-79 through 1993-94. Union County has been the most frequent and consistent site of avian cholera outbreaks in the Mississippi Flyway, with mortality documented in 8 of 16 years between 1978-79 and 1993-94 (Table 1). There was a 4-year hiatus between 1980-81 and 1985-86, and no outbreaks have been recorded in southern Illinois since 1989-90. Outbreaks were

Table 1. Documented occurrences of avian cholera mortality in waterfowl within the Mississippi Flyway from January 1979 through March 1994[a].

Population Range Location	1978/ 1979	1979/ 1980	1980/ 1981	1985/ 1986	1986/ 1987	1987/ 1988	1988/ 1989	1989/ 1990	1990/ 1991	1991/ 1992	1992/ 1993	1993/ 1994
Mississippi Valley Population												
Union County CA, IL	ac	b	c	c	cd	e	cd	cd	-	-	-	-
Horseshoe Lake CA, IL	ac	-	-	-	cd	-	cd	cd	-	-	-	-
Rend Lake WMA, IL	-	-	-	-	-	-	-	cd	-	-	-	-
Ballard Co. WMA, KY	-	-	c	-	-	cd	-	cd	-	-	-	-
East-Central, WI	-	bc	-	-	-	-	-	-	-	-	-	-
Eastern Prairie Population												
Eskimo Point, NT, Canada	-	b	-	-	-	-	-	-	-	-	-	-
Manitou, MB, Canada	-	b	-	-	-	-	-	-	-	-	-	-
Oak Hammock, MB, Canada	-	-	-	-	-	-	-	-	-	-	-	d
Lac qui Parle WMA, MN	-	-	-	-	-	-	-	cd	-	d	-	d
Big Stone NWR, MN	-	-	-	-	-	-	-	d	-	d	-	-
De Soto NWR, IA	-	b	-	-	d	-	d	d	-	d	-	-
Riverton WMA, IA	-	b	-	-	d	d	-	d	d	d	-	-
Squaw Creek NWR, MO	-	b	-	-	-	-	-	-	d	d	-	-
Swan Lake NWR, MO	-	bc	c	-	-	-	-	cd	-	-	-	-

Table 1. Continued.

Population Range Location	1978/ 1979	1979/ 1980	1980/ 1981	1985/ 1986	1986/ 1987	1987/ 1988	1988/ 1989	1989/ 1990	1990/ 1991	1991/ 1992	1992/ 1993	1993/ 1994
Bob Brown, WMA, MO	-	-	-	-	-	-	-	-	-	d	-	-
Brookfield, WMA, MO	-	c	-	-	-	-	-	c	-	-	-	-
Montrose WMA, MO	-	-	-	-	-	-	-	cd	-	-	-	-
Sumner, MO	-	-	-	-	-	-	-	-	-	-	-	d
Slater, MO	-	-	-	-	-	-	-	cd	-	-	-	-
Grand Pass WMA, MO	-	-	-	-	-	-	-	-	d	-	-	-
St. Louis, MO	-	-	-	-	-	-	-	d	-	-	-	-
Lacassine NWR, LA	-	-	-	-	-	-	d	-	-	-	-	-

[a] Sources: a = Windingstad et al. (1983); b = Brand (1984); c = Windingstad et al., (in press); d = quarterly mortality reports appearing in supplements to The Journal of Wildlife Diseases; e = unpublished data, SIUC Cooperative Wildlife Research Laboratory.

sporadic on other southern Illinois wintering areas; 4 occurred at Horseshoe Lake, and only 1 at Rend Lake WMA through 1993-94. Three outbreaks were documented at Ballard County WMA during the same period. Mortality due to avian cholera has never been detected at Crab Orchard NWR where large numbers of MVP geese also winter.

No outbreaks were recorded on wintering areas of MVP geese in southern Illinois or western Kentucky after 1989-90. After widespread outbreaks in the Mississippi Flyway during 1989-90, disease foci have shifted away from the range of MVP geese, and become more prevalent on wintering and migration areas of EPP geese. Outbreaks have occurred near Lac qui Parle MN, Oak Hammock MB, and at Squaw Creek National Wildlife Refuge (NWR) in 3 of 4 years since the winter of 1989-90. No waterfowl mortality due to avian cholera was reported in southern Illinois during this study.

Southern Illinois.--Waterfowl mortality from avian cholera has not been observed before 25 December, or after 9 March, in southern Illinois (Table 2). This period corresponded with the most severe winter weather and highest densities of Canada geese on southern Illinois wintering areas during most years. Of 13 outbreaks that occurred between 1979 and 1990, 8 were associated with periods of below normal temperatures. However, mortality was apparently greatest for outbreaks that occurred during cold weather (Windingstad et al. In Press). Minimum estimated mortality due to avian cholera tended to be greater when larger numbers of geese were present during outbreaks.

The first Canada geese diagnosed with avian cholera during the 1978-79 outbreak were collected on Barker Lake (U6; Fig. 1) at Union County CA (Windingstad et al. 1983).

Table 2. Description of avian cholera outbreaks in MVP Canada geese on southern Illinois wintering areas[1]. Information was compiled from Windingstad et al. (1983, In Press), Brand (1989) and unpublished data (Ill. Dept. Conserv., SIUC Coop. Wildl. Res. Lab.).

Location	Dates	Estimated minimum mortality	Population size	Weather conditions associated with outbreak	Outbreak foci
UCCA	01/16/79 to 02/09/79	750	30,000	Below normal temps. w/snow and ice cover; followed by warming and snow/ice melt.	Barker Lake
HLCA	02/09/79 to 02/15/79	13	30,000	Below normal temps. w/snow and ice cover; followed by warming and snow/ice melt.	Island swim-in trap site
UCCA	01/19/81 to 02/12/81	71	50,000	Below normal temps., no snow.	Not reported
UCCA	01/29/86 to 02/06/86	450	39,000	Below normal temps, trace of snow cover.	Not reported
UCCA	01/13/87 to 02/20/87	1,500	46,000	Seasonal temperatures, trace of snow cover.	Not reported
HLCA	02/15/87 to 02/20/87	300	45,000	Above normal temps., no snow reported or ice cover.	Not reported
UCCA	02/13/88 to 02/14/88	25	90,000	Cold front with freezing temps. followed by rapid warming.	Barker Lake, levee borrow pit
HLCA	01/23/88 to 02/02/88	9	55,000	Cold front with freezing temps. followed by rapid warming.	Widespread in crop fields on thumb, island, and the Public Hunting Area.
UCCA	01/09/89 to 03/09/89	452	120,000	Above normal temps., little precipitation, followed by cold front with snow/ice storm	Barker, Grassy, and Lyerla Lakes; other shallow wetlands.
HLCA	01/11/89 to 02/08/89	1,200	300,000	Above normal temps., light precipitation.	Shallow wetlands and and lake shore on the Island.
UCCA	12/25/89 to 01/15/90	6,000	190,000	Outbreak followed 10 days of extreme cold temps. Lakes and wetlands frozen, trace of snow.	Barker, Lyerla, and Grassy Lakes, office ponds.
HLCA	01/01/90 to 02/02/90	1,000	360,000	Below normal temps., no snow freezing conditions.	Small open water areas on Horseshoe Lake.
RLWMA	01/18/90 to 02/01/90	<500	170,000	Mild temps., no snow.	Rend Lake shoreline on the refuge area.

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[1] UCCA = Union County Conservation Area, HLCA = Horseshoe Lake Conservation Area, RLWMA = Rend Lake Wildlife Management Area.

Most mortality during subsequent outbreaks was recorded on and near Barker Lake, with generally fewer numbers of dead geese collected on Grassy (U1) and Lyerla (U14) Lakes and shallow wetlands (U2-U5) within Union County CA (Illinois Dept. Conserv., unpubl.). Outbreaks tended to occur primarily around the island (H1 and H6) at Horseshoe Lake CA (Fig. 2) where the swim-in trap site (H2) was the focus of most outbreaks (Illinois Dept. Conserv., unpubl.).

Outbreaks were generally most severe at Union County CA, and with one exception, preceded outbreaks at Horseshoe Lake CA. Cholera outbreaks have never been reported at Crab Orchard NWR, and only once at Rend Lake WMA where roosting and resting sites used by geese are mostly large, relatively deep lakes (Raveling 1969, Anderson and Joyner 1985, Caithamer et al. 1992). The low incidence or lack of avian cholera outbreaks at Rend Lake and Crab Orchard could be due to lower densities of birds, and/or dilution of the disease organism in the water column where birds concentrate during fall and winter. At Horseshoe Lake and Union County CA, shallow wetlands comprised 16% and 41%, respectively, of nonforested wetland and lake surface areas available to geese. Consequently, there was typically high use of shallow wetland sites by roosting and resting geese on these 2 areas, particularly at Union County CA (Caithamer et al. 1992, Moritz 1992). Additionally, winter populations of geese at Crab Orchard and Rend Lake decline during most years as geese disperse to areas farther south (Horseshoe Lake and Union County CA, Ballard County WMA) during mid-winter when most outbreaks have occurred. Thus, if there is a single pattern to avian cholera outbreaks in southern Illinois, it is that they tend to be more frequent and more severe in areas with relatively small shallow wetlands such as Union County CA.

Occurrence of the disease at Rend and perhaps Horseshoe lakes appears to be associated with movement of infected birds into these areas from Union County CA.

Outbreak Monitoring

Horseshoe Lake Conservation Area.--Waterfowl (primarily Canada geese) used mostly lake sites at Horseshoe Lake CA during 1991-92, with only sporadic use of shallow wetlands on the island. Lake sites H1, H6, and H9 received consistently high use until the end of January 1992, after which warm weather prompted the departure of many geese (Table 3). Site H6 generally had the highest waterfowl densities, frequently exceeding 200 birds/ha (maximum = 1,122 birds/ha). Lake sites H5A and H5B, however, did not receive substantial use until mid-January, but held birds in relatively large numbers until the end of February when surveys were discontinued. Few geese were observed using wetland site H2; wetlands H3 and H4 were used mostly during the first 2 weeks of February 1992. Waterfowl densities on wetland H3 exceeded 950 birds/ha during early to mid-February. Only 3 wetland or lake sites were routinely monitored at Horseshoe Lake CA during 1992-93 (Table 3), when water samples were collected for isolation of P. multocida (see Job 2, Environmental and Biological Factors). Densities of waterfowl observed using wetland site H2 (swim-in trap site) in 1992-93 were considerably higher than recorded the previous year.

Although bald eagles (Haliaeetus leucocephalus) rapidly consumed carcasses, evidence of mortality due to any cause was light during winters 1991-92. Only 10 Canada geese were found dead, and all were found on lake sites before the end of January. All of 5 carcasses

Table 3. Densities (birds/ha) of waterfowl observed on selected wetland and lake sites at Union County and Horseshoe Lake Conservation Areas in southern Illinois during October - February 1991 - 1994.

Wetland		1991-92			1992-93			1993-94		
Site	Type	Mean	SD	Max.	Mean	SD	Max.	Mean	SD	Max.
Horseshoe Lake										
H1	Lake	18	20	82	64	73	185			
H2	Shallow	<1	<1	2	677	1,363	3,704			
H3	Shallow	115	282	1,011						
H4	Shallow	37	117	500						
H5A	Lake	90	215	1,019						
H5B	Lake	75	86	399						
H6	Lake	164	273	1,122	132	96	233			
H9	Lake	45	90	354						
Union County										
U1N	Shallow	80	195	759	29	31	107	115	174	676
U1S	Lake	65	81	273	82	240	1,099	40	87	406
U2N	Shallow	247	434	2,027	226	322	1,294	367	1,171	7,611
U2S	Shallow	348	1,244	6,385	397	1,002	3,987	147	278	1,236
U3	Shallow	516	1,050	5,363	448	656	2,144	198	551	3,570
U4	Shallow	9	36	188	3	5	20	21	54	289
U5N	Shallow	36	87	433	13	25	105	12	47	262
U5S	Shallow	229	415	1,858	33	60	239	210	379	1,453
U6	Shallow	84	56	200	20	19	68	219	512	2,803
U8	Shallow	84	132	480	20	36	133	22	57	268
U14	Lake	8	16	79	6	7	30	1	4	22

recovered for necropsy showed evidence of death due to trauma (hunter crippling). We documented no mortality due to avian cholera in waterfowl at Horseshoe Lake CA during this study.

Union County Conservation Area.--The largest numbers of waterfowl (primarily Canada geese) were usually observed at Grassy Lake (U1), however, groups of >1,000 birds were commonly observed at Lyerla Lake (U14), Barker Lake (U6), and the Windmill Pond (U3) during 1991-92. The highest waterfowl densities were typically observed on shallow wetland sites (U1N, U2-6, and U8) compared to the Grassy (U1S) and Lyerla (U14) Lake sites (Table 3). Greater waterfowl also were generally observed at Union County CA (maximum = 79 - 6,385 birds/ha) compared to Horseshoe Lake CA (maximum = 2 - 1,122 birds /ha). The highest waterfowl densities (>400 birds/ha) were associated with shallow wetlands, but not lakes. The tendency toward crowding of geese on shallow wetlands may play an important role in disease outbreaks at Union County CA. During winters 1991-92 and 1992-93, 7 of 11 geese found dead were in shallow wetlands, reflecting greater use of shallow wetlands at Union County CA, compared to Horseshoe Lake CA.

Similar to 1991-92, greater waterfowl densities (primarily Canada geese) generally occurred on shallow wetland sites (maximum = 20 - 7,611 birds/ha) than on lake sites (maximum = 22 - 1,099 birds/ha) during 1992-93 and 1993-94 (Table 3). Waterfowl densities on individual wetland and lake sites were variable among years, with lower maximum densities occurring in 1992-93 compared to 1991-92 and 1993-94. Large concentrations of geese on individual wetlands over long periods of time were not observed in 1992-93, and geese were more dispersed compared to winters 1991-92 and 1993-94. This could be attributed to a relatively mild winter in 1992-93 when none of the wetland sites were frozen during the monitoring period. Winter 1993-94 was more severe, with subnormal temperatures and snow/ice cover that persisted during late December through mid-January. Wintering geese in southern Illinois tend to spend more time on roosting areas when daytime temperatures are low and snow or ice cover is extensive (Caithamer et al. 1992). Freezing of shallow wetlands tends also to concentrate geese on larger lakes, resulting in local crowding around small areas of open water. These conditions likely caused generally higher densities of geese on wetlands that we monitored in 1993-94 (22 - 7,611 birds/ha) compared to 1992-93 (20 - 3,987 birds/ha).

Despite regular and intensive monitoring, we detected no evidence of waterfowl mortality due to infectious disease during winters 1991-92 through 1993-94 at Union County CA. Very few dead or moribund geese were observed throughout these 3 winters, consequently, only small numbers of carcasses were recovered for necropsy. Avian cholera was not detected in any geese recovered in a condition suitable for necropsy.

Job 2: Environmental and Biological Factors

Objective: Determine environmental, physical, and biological factors associated with avian cholera outbreaks, and specific areas where the disease appears endemic.

INTRODUCTION

Environmental Persistence

P. multocida can persist for variable time periods in wetland water and soil. Price and Brand (1984) isolated virulent P. multocida from water during, and for several days after, an avian cholera outbreak in the Rainwater Basin of Nebraska. Backstrand and Botzler (1986) isolated P. multocida from pond water 3 and 10 days after an epornitic, but failed to isolate the organism after 1 day when inoculated into sterilized and non-sterilized pond water. Detectable concentrations of P. multocida were present < 20 days in inoculated soil samples. Known survival of P. multocida in soil ranges from several to 240 days, depending on ambient temperature, presence of animal protein and other undetermined factors (Rosen and Bischoff 1950, Olson and Bond 1968, Awad et al. 1976, Backstrand and Botzler 1986). Backstrand and Botzler (1986) and Price and Brand (1984) concluded that infected carcasses were the primary sources of P. multocida present in water during and after disease outbreaks.

Previous studies indicated that survival of P. multocida in water is influenced by physical and chemical characteristics of the medium. Bredy and Botzler (1989) found that the presence of soluble proteins and sodium chloride enhanced survival in water, and that the organism survived better in water at 18 C than at 2 C. They also detected P. multocida >1 year after inoculating the organism into water. Windingstad et al. (1984) and Gordon (1989) found that concentrations of Ca and Mg were higher in wetlands located in the western Rainwater Basin where 92% of mortality due to avian cholera was documented during 1975-83, than in the eastern portion where only 8% of mortality occurred. These findings prompted Price et al. (1992) to investigate the effects of specific ion concentrations on survival of P. multocida in water. They found that survival was enhanced by high concentrations of calcium and/or magnesium. Bredy and Botzler (1989) found that survival of P. multocida in water was enhanced in the presence of animal protein, and temperatures of 18 to 20 C.

Culture and Isolation From the Environment

Despite findings that P. multocida may survive > 1 year under specific circumstances in water (Bredy and Botzler (1989), and for up to 240 days in soil (Rosen and Bischoff 1950), the possible role of water and soil as year-round reservoirs of the disease agent has not been clearly defined. The difficulty of isolating P. multocida from environmental samples has hindered clear understanding of the role of the physical environment as a bacterial reservoir for avian cholera epornitics. The most commonly used method to detect P. multocida in water requires intraperitoneal inoculation of samples into mice, followed by post-mortem liver and lung culture on 5% sheep blood agar and/or Rosen's dextrose starch agar (Price and Brand 1984). No other reliably tested methods are currently available to detect low numbers of P. multocida in environmental samples, although promising results might be expected in the future from use of the polymerase chain reaction (PCR) to detect bacterial pathogens (Atlas and Bej 1990).

In the absence of reliably tested PCR techniques, growth and isolation on selective media developed and tested for use with water, sediment, and soil samples may be the most effective method of detecting P. multocida in environmental samples. The NWHRC previously formulated selective enrichment broth and solid selective medium for isolation of Pasteurella multocida (Type I) from contaminated avian tissue samples, but the efficacy of these media in recovering P. multocida from water had not been evaluated. A cooperative project was initiated with NWHRC to determine the efficacy of selective enrichment broth and solid selective media in detecting low concentrations of P. multocida in samples of pond water contaminated with natural bacterial flora. The objectives of this work were to determine minimum concentrations of P. multocida that can be isolated with varying degrees of reliability using the selective media, and to determine optimum incubation time that will permit growth of P. multocida while inhibiting growth of other bacteria. This work was reported by Moore et al. (1994) and is summarized briefly in this report.

We planned to use the selective media to attempt isolation of P. multocida from selected wetlands and suspected enzootic sites at Union County and Horseshoe Lake CA before, during, or after an epornitic of avian cholera. Isolation of the organism in the absence of the outbreak would lend support to the environmental persistence hypothesis that epornitics of avian cholera are initiated when susceptible birds are exposed to virulent P. multocida and become infectious after arriving at an enzootic site.

METHODS

Water Quality Sampling

Physical and chemical characteristics of surface water were measured at Horseshoe Lake CA on 10 March 1992 and at Union County CA on 11 March 1992. Measurements were recorded at 2 locations within each of 8 preselected wetland or lake sites on both Conservation Areas, for a total of 32 samples. One sample within each wetland or lake site was taken from a near-shore area, the other was taken off-shore.

Measurements of surface water quality were also obtained from 5 wetland or lake sites at Union County and Horseshoe Lake CAs on 20 October 1992. Measurements were obtained for 2 locations within each wetland or lake site, for a total of 20 samples. There were no differences in water quality measurements between near- vs. off-shore areas in March 1992, so sample locations in fall 1992 were distributed within representative areas of wetland or lake sites without regard to distance from shore.

Water samples were collected during March and October 1992 for in vitro chemical analyses by submerging 2 polyethylene bottles (0.5 l) to depths of 5-10 cm. Two ml of concentrated sulfuric acid were added to one of the bottles to preserve samples for ammonia determination. Samples were stored on ice and sent to A&L Analytical Laboratories (Memphis, TN) for quantification of sodium, calcium, magnesium, chloride, sulfate, nitrate, and ammonia concentrations.

Conductivity, pH, dissolved oxygen, and temperature were measured in situ using a Yellow Springs Instrument (YSI) Company Model 33 salinity-conductivity-temperature meter, a YSI Model 57 oxygen meter, and a Cole-Parmer Model 5941 pH tester during

March and October 1992. Measurements of pH, conductivity, dissolved oxygen, and temperature also were recorded in conjunction with collection of water samples to attempt isolation of P. multocida on 16 November and 1 December 1992. No in vitro chemical analyses were conducted during these sample periods.

Combining years, water quality measurements provided a profile of physical and chemical parameters for representative wetland and lake sites at Horseshoe Lake and Union County CAs. These values were compared with those published for other enzootic sites in North America where similar measurements were obtained.

Statistical analyses of water quality parameters measured in March 1992 were conducted using the General Linear Models (GLM) procedure of the Statistical Analysis System (SAS Institute 1985). Analysis of variance (ANOVA) was used to compare physical and chemical water quality parameters between locations (Union County vs. Horseshoe Lake), wetland types (shallow wetlands vs. lakes and deeper impoundments), and sites within wetlands (near- vs. off-shore areas). Shallow wetlands were differentiated from lakes and deeper impoundments (i.e. borrow pits) as described in Job 1. Duncan's multiple range test was used to separate differences among means for significant main effects for independent variables containing > 2 classes. Differences were considered significant at $P \leq 0.05$.

Price et al. (1992) demonstrated that concentrations of Ca and Mg influence the survival of P. multocida in water. Electrical conductivity in aquatic systems is a direct function of the total amount of dissolved solids. Since Ca and Mg were the principal cations present in wetlands at both Conservation Areas, we used multiple linear regression to develop prediction equations for estimating Ca and Mg concentrations from in situ measurements of total conductivity. Data from March and October sample periods were pooled for statistical analyses, providing data for 52 water samples (32 from March and 20 from October 1992).

Culture and Isolation of P. multocida

Selective media testing.--The ability of selective media previously developed by NWHRC to inhibit growth of contaminant organisms found in environmental samples was initially tested using water and wetland sediments collected on 31 May 1992. Samples were collected at sites 1B (Grassy Lake) and 6B (Barker Lake) at Union County CA (Figure 1), and sites 1A (Horseshoe Lake) and 7B (shallow pond; Figure 2) at Horseshoe Lake CA. These wetland sites were selected because they had the highest and lowest Ca concentrations found in water samples collected in March 1992.

Water samples were collected by submerging 0.5 l polyethylene bottles to depths of 1-2 cm below water surface. Sediment samples were taken by submerging 0.5 l polyethylene bottles to the wetland bottom before they were opened to collect surface sediments. Samples were transported on wet ice to NWHRC in Madison, WI.

Growth of several known isolates on blood agar, P. multocida (PM) selective media, and Rosen's agar was compared before testing environmental samples. Organisms tested for growth included Escherichia coli, P. multocida, Proteus vulgaris, Pseudomonas aeruginosa, Salmonella typhimurium, Staphylococcus aureus, and Streptococcus pneumoniae. All samples were first incubated in PM selective enrichment broth to enhance growth of P.

multocida. Samples were not directly plated onto solid media except during initial identification of organisms in the samples tested.

All water and substrate samples were cultured on 5% sheep blood agar, Rosen's dextrose starch agar, and PM selective solid media to detect natural bacterial flora. Isolated bacteria were Gram stained and Gram negative samples were identified by the API 20E system (Analytab Products, Plainview, NY).

Ability to recover colonies from different dilutions of inoculated P. multocida was tested with 2 water samples, 2 substrate samples, and a broth control. A 4 ml aliquot was taken from each of the 2 water samples and broth control and distributed to sterile tubes. Two ml aliquots from the 2 substrate samples were mixed with 2 ml of 0.85% NaCl to facilitate handling. Twelve ml of PM selective enrichment broth was added to each sample. After mixing, aliquots of broth mixture were taken from each tube and distributed among 8 sterile tubes in equal volumes of 1.8 ml/tube. Different serial dilutions of P. multocida were added to 7 of the 8 tubes ranging from 10^0/ml to 10^6/ml dilutions. Actual P. multocida titers at these dilutions ranged from 4 X 10^{-2} to 4.2 X 10^4 colony forming units (CFU)/ml. The 8th tube was a control to which P. multocida was not added. Tubes were swabbed at 9, 16, and 24 hours then plated on blood agar, Rosen's agar with PPLO serum, and PM selective medium. Plates were examined to detect the presence of P. multocida colonies after incubation for 24 hours at 37 C in 5% CO_2.

A more intensive experiment was conducted during May 1993 to evaluate 4 different culture and isolation procedures using PM selective media, and to determine the probability of recovering P. multocida from water and tissue samples at different concentrations of the organism. Four different isolation procedures using blood agar or selective media were tested for isolation of *P. multocida* from pure culture and from water contaminated with natural bacterial flora. Isolation procedures that were evaluated included: 1) direct plating on standard blood agar; 2) direct plating on PM selective media; 3) inoculation into PM selective enrichment broth followed by plating on blood agar; and 4) inoculation into PM selective enrichment broth followed by plating on PM solid selective medium. Reliability of these procedures was compared by determining recovery of the organism from 25 replicate sample sets at 5 concentrations of *P. multocida* ranging from 0 to 6 X 10^3 CFU/ml. A stock culture of *P. multocida* Serotype I provided by NWHRC was used to prepare dilutions of 6 X 10^0, 6 X 10^1, 6 X 10^2, and 6 X 10^3 CFU/ml. Actual concentrations of *P. multocida* in dilution bottles were determined by duplicate spread-plate titrations prepared after adding *P. multocida* and again at the end of the experiment.

One set of dilutions was prepared in brain heart infusion broth (BHIB) and another in water collected from Barker Lake (U6) at Union County CA. Uninoculated BHIB and lake water were used as controls. Twenty-five swabs from each set of 2 dilutions and 5 concentration levels (4 dilutions plus a control) of *P. multocida* were streaked on selective media plates (*n* = 250). An identical set of swabs (*n* = 25) were streaked on blood agar (blood agar base, DIFCO; 5% defibrinated sheep blood, Remel, Lenexa, KS) plates (*n* = 250). Aliquots of 0.5 ml from each set of dilutions and each concentration level of *P. multocida* were inoculated into 25 tubes, each containing 5 ml of selective enrichment broth (*N* = 250). Selective enrichment broth tubes were incubated for 16 - 18 hours at 37 C in 5% CO_2. Swabs from each broth tube were cultured on selective media and blood agar plates after incubation. These plates were incubated for 24 hours at 37 C in 5% CO_2. Plates were examined after incubation for growth of P. multocida. Suspect colonies from plates inoculated with water collected from wetlands were further evaluated, first with MacConkey agar and then with the API 20E system. More detailed description of laboratory procedures and media formulations for this experiment are found in Moore et al. (1994).

Environmental sampling.--Water samples were collected from selected wetland and lake sites at Horseshoe Lake and Union County CAs to test for the presence of P. multocida at locations with histories of avian cholera outbreaks. Samples were collected 7 times during October 1992 through February 1993. We sampled surface water because that is where susceptible hosts were most likely to become infected during an outbreak. Ten samples were collected at Union County CA, during each sampling period from Grassy Lake (U1), Headquarters Ponds (U2), and Barker Lake (U6) (Fig. 1). Water was collected from wetlands H1 ($n = 5$), H6 ($n = 5$), and H2 ($n = 10$) at Horseshoe Lake CA (Fig. 2) during each sampling period. Numbers of waterfowl using these wetland and lake sites were recorded prior to collection of water samples. Water samples were collected from locations within wetlands used by geese at the time of sampling, and where surface particles accumulated, to enhance the probability of detecting P. multocida, if present.

We collected additional water samples from Barker Lake (U6) at Union County CA to detect presence of P multocida during 1993-94. Surface water was sampled on the north end of the lake where densities of waterfowl were greatest during fall and winter. Twenty samples were collected from this area on 27 October 1993; 6 samples were collected on 19 January 1994.

About 200 ml of surface water was collected per sample in sterile glass bottles. Water samples were kept at 2 - 10 C until they arrived in the laboratory. All samples were immediately cultured on selective media after arrival in the laboratory (< 8 hours). One ml aliquots from each water sample were inoculated into 2 tubes with selective enrichment broth. Enrichment broth tubes were incubated for 16 hours at 37 C in 5% CO_2. Samples from selective enrichment tubes were inoculated by swabbing onto PM solid selective medium plates after incubation. These plates were incubated 24 - 48 hours at 37 C in 5% CO_2. After incubation on selective media, well isolated colonies suspected to be P. multocida were subcultured on blood agar plates and incubated for 24 hours at 37 C in 5% CO_2. After incubation, bacterial colony growth, morphology, pigment, and action on blood cells was examined. Suspect P. multocida colonies were further evaluated by oxidase test and Gram stain. Isolates that were Gram negative and oxidase positive were inoculated again onto triple sugar iron slants (TSI) on MacConkey plates (Killian et al. 1981). TSI slants and MacConkey plates were then incubated for 24 h at 37 C. Isolates that produced acid reaction in slant and butt of TSI slants, and did not grow on MacConkey agar were further analyzed by the API 20E system (Sherwood Medical Company, St. Louis, MO).

RESULTS AND DISCUSSION

Water Quality

Spring 1992.--There was substantial variation in temperature and dissolved oxygen among wetland and lake sites at Horseshoe Lake CA on 10 March 1992 (Table 4). A sudden cold front lowered air temperatures to 0 C, and winds exceeded 32 kph for most of

Table 4. In situ measurements of water quality from individual wetland sites on Horseshoe Lake and Union County Conservation Areas during March 1992.

Wetland Site	Temperature (°C)	Conductivity (micromhos/cm^2)	Dissolved Oxygen (mg/l)	pH
Horseshoe Lake				
H1A	9.0	102	8.3	7.3
H1B	11.0	80	6.8	7.2
H2A	3.0	50	14.0	8.3
H2B	3.0	52	14.0	8.3
H3A	7.0	50	11.2	7.2
H3B	4.0	50	11.8	7.2
H4A	3.0	40	12.4	6.9
H4B	2.5	40	12.0	7.0
H5A	10.5	62	6.5	7.0
H5B	12.0	62	8.2	6.9
H6A	11.5	75	6.2	7.0
H6B	12.0	73	9.4	7.4
H7A	6.5	40	11.2	7.7
H7B	6.5	40	11.4	7.6
H8A	9.0	29	9.4	7.1
H8B	9.5	38	8.2	7.0
Union County				
U1A	5.5	54	12.2	7.2
U1B	4.5	54	11.6	8.3
U3A	4.5	268	11.6	8.1
U3B	5.0	271	13.6	8.1
U5A	5.0	280	16.2	8.0
U5B	5.0	270	14.0	8.0
U6A	7.0	220	10.8	7.7
U6B	7.5	215	15.0	7.9
U7A	8.0	385	11.2	7.5
U7B	9.5	295	11.7	7.7
U10A	9.0	60	9.8	7.3
U10B	8.0	60	9.1	7.4
U12A	7.0	200	12.6	7.9
U12B	8.0	205	13.1	8.2
U14A	9.0	62	13.4	8.3
U14B	10.0	67	13.5	8.5

the day. These weather conditions affected water quality measurements of small, shallow wetlands to a greater degree than in the main body of Horseshoe Lake. Mean temperature in shallow wetland sites (H2-H4 and H7) was 4.4 C (SD = 1.9), compared to 10.6 C (SD = 1.3) in 4 lake sites (H1, H5, H6, H8). Wave action caused high oxygen levels ($\bar{x}$ = 12.3 mg/l, SD = 1.2) in shallow wetlands, compared to lake sites ($\bar{x}$ = 7.9 mg/l, SD = 1.2). Conductivity was low (38-102 μmhos/cm^2) in all samples. Measurements of pH recorded in all wetland and lake sites were near neutral (6.9-8.3).

No consistent differences in temperature and dissolved oxygen were observed among wetland and lake sites at Union County CA, but extremely high dissolved oxygen levels (sometimes supersaturated) occurred at sites U3B, U5A, U5B, and U6B (Table 4). This was due to the formation of a thin sheet of ice at these locations over-night, preventing release of oxygen that had been mixed into the water by high winds on the previous day.

In vitro analyses indicated that Ca and SO_4 were the principal dissolved ions present in wetland and lake sites at Horseshoe Lake CA, reaching concentrations up to 22 mg/l (Table 5). Na, Mg, Cl, NO_3 , and NH_3-N concentrations were mostly < 5 mg/l, except for NH_3-N at 2 wetland sites (see below). Water chemistry profiles of wetland and lake sites at Union County CA were similar to Horseshoe Lake CA, except that Mg was an important constituent of dissolved ion content in addition to Ca and SO_4 (Table 5). Concentrations of NO_3 and NH_3-N were very low, with levels that were below detection limits at most wetland and lake sites. Despite its relatively large surface area, Barker Lake (U6) had a water chemistry profile more similar to smaller shallow wetlands than to larger or deeper wetlands and lakes at Union County CA. Characteristics that Barker Lake shared in common with

Table 5. In vitro measurements of water quality from individual wetland and lake sites on Horseshoe Lake and Union County Conservation Areas during March 1992.

Wetland Site	Sodium (mg/l)	Calcium (mg/l)	Magnesium (mg/l)	Chloride (mg/l)	Sulfate (mg/l)	Nitrate (mg/l)	Ammonia (mg/l)
Horseshoe Lake							
H1A	5	22	7	4	22	<0.10	<0.5
H1B	4	16	4	4	15	<0.10	<0.5
H2A	1	16	2	2	14	0.16	0.8
H2B	1	17	2	<2	15	0.43	0.8
H3A	4	3	2	4	16	1.43	9.3
H3B	4	3	1	6	21	1.08	10.0
H4A	6	3	1	8	21	1.48	7.8
H4B	6	2	<1	7	17	1.48	8.1
H5A	3	12	3	4	15	<0.10	<0.5
H5B	3	12	3	2	15	<0.10	<0.5
H6A	3	15	4	<2	17	<0.10	<0.5
H6B	3	14	3	4	18	0.21	<0.5
H7A	<1	9	3	<2	13	0.81	0.6
H7B	<1	9	3	<2	14	0.91	<0.5
H8A	1	6	2	2	12	0.78	0.6
H8B	1	7	2	2	12	0.57	<0.5
Union County							
U1A	2	15	3	<2	14	<0.10	<0.5
U1B	2	14	3	<2	14	<0.10	<0.5
U3A	5	66	24	2	18	<0.10	<0.5
U3B	5	66	4	2	18	0.15	<0.5
U5A	5	61	25	2	27	0.13	<0.5
U5B	5	61	25	4	26	0.24	0.5
U6A	3	50	14	2	17	0.40	0.6
U6B	2	47	13	<2	15	0.16	0.6
U7A	5	82	25	2	14	<0.10	<0.5
U7B	5	83	27	4	15	0.23	<0.5
U10A	1	12	4	<2	12	0.10	<0.5
U10B	1	12	3	<2	13	0.39	<0.5
U12A	4	49	15	2	14	<0.10	<0.5
U12B	4	49	15	<2	14	<0.10	<0.5
U14A	1	15	3	<2	10	<0.10	<0.5
U14B	1	15	3	2	10	<0.10	<0.5

other shallow wetlands included: receiving direct surface runoff from adjacent cropland, dewatering during the summer, and pumping of ground-water to maintain open roosting sites for geese during winter. These characteristics were not entirely shared by other deep wetland or lake sites at Union County CA.

No differences in in vitro measurements of water quality were observed between Horseshoe Lake and Union County CA ($\underline{P} < 0.05$) when all wetland types were combined (Table 6). Likewise, there were no differences detected between shallow wetlands and deeper impoundments and lakes when Horseshoe Lake and Union County sites were pooled. However, there was an interaction of location with wetland type for Ca, Mg, and NO_3 ($\underline{P} < 0.05$). Conductivity, Ca, and Mg levels were higher at Union County CA than at Horseshoe Lake CA during March 1992 (Table 6). These differences were attributable to higher total conductivities and concentrations of Ca and Mg in shallow wetlands at Union County CA (Table 6). Union County lakes and deeper wetlands had conductivity levels and concentrations of Ca and Mg that were similar to shallow wetlands and lakes at Horseshoe Lake CA. Elevated concentrations of these basic cations in shallow wetlands at Union County CA may have been due to: 1) a higher proportion of surface water drainage received by shallow wetlands at Union County originated from crop fields, whereas lakes were generally surrounded by forest cover, or 2) greater soil/water mixing and turbidity in shallow wetlands caused by surface run-off or wave action. However, these same conditions would also apply to shallow wetlands at Horseshoe Lake CA, where similar differences were not observed. Differences in water quality between shallow wetlands at Union County and

Table 6. Mean measurements of water quality parameters of wetland and lake sites on Horseshoe Lake and Union County Conservation Areas by location, wetland type, and wetland types by location during March 1992.

Comparison		Na (mg/l)	Ca (mg/l)	Mg (mg/l)	Cl (mg/l)	SO_4 (mg/l)	NO_3 (mg/l)	NH_3-N (mg/l)	Temp. (°C)	Conductivity (micromhos/cm^2)	Dissolved O_2 (mg/l)	pH
WETLANDS AND LAKES COMBINED, HLCA VS. UCCA												
Horseshoe Lake	MEAN	2.81	10.38	2.63	3.06	16.06	0.58	2.38	7.50	55.19	10.06	7.32
	SD	1.91	5.86	1.54	2.44	3.01	0.55	3.75	3.41	18.72	2.46	0.43
Union County	MEAN	3.19	43.56	14.13	1.38	15.69	0.11	0.11	7.03	185.38	12.46	7.88
	SD	1.67	25.09	9.49	1.36	4.66	0.14	0.22	1.82	106.07	1.79	0.37
BOTH AREAS COMBINED, WETLANDS VS. LAKES												
LAKES	MEAN	2.21	13.36	3.36	1.71	14.21	0.15	0.04	9.32	62.71	9.47	7.42
	SD	1.26	3.73	1.17	1.67	3.12	0.24	0.15	2.13	16.97	2.32	0.52
WETLANDS	MEAN	3.61	37.56	12.28	2.61	17.17	0.50	2.17	5.67	165.06	12.66	7.74
	SD	1.92	28.51	10.25	2.38	4.00	0.53	3.58	2.01	114.19	1.48	0.42
INTERACTION OF LOCATION AND IMPOUNDMENT TYPE												
HLCA, LAKES	MEAN	2.88	13.00	3.50	2.75	15.75	0.20	0.08	10.56	65.13	7.88	7.11
	SD	1.27	4.77	1.50	1.39	3.07	0.29	0.20	1.18	21.80	1.17	0.16
HLCA, WETLANDS	MEAN	2.75	7.75	1.75	3.38	16.38	0.97	4.68	4.44	45.25	12.25	7.53
	SD	2.38	5.67	0.97	3.12	2.91	0.46	4.18	1.78	5.29	1.08	0.51
UCCA, LAKES	MEAN	1.33	13.83	3.17	0.33	12.17	0.08	0.00	7.67	59.50	11.60	7.83
	SD	0.47	1.34	0.37	0.75	1.67	0.14	0.00	1.99	4.54	1.67	0.54
UCCA, WETLANDS	MEAN	4.30	61.40	20.70	2.00	17.80	0.13	0.17	6.65	260.90	12.98	7.91
	SD	1.00	12.56	5.35	1.26	4.60	0.13	0.26	1.60	52.70	1.66	0.21

Horseshoe Lake CA could be due to differences in soil fertility, amounts of surface run-off due to soil texture or permeability differences, or differences in water pumped to flood wetlands.

Nitrate levels of shallow wetlands at Horseshoe Lake CA were higher than in Horseshoe Lake, and also higher than in and lakes and shallow wetlands at Union County CA. No differences in nitrate concentrations were observed between Union County CA shallow wetlands and lakes. Some of the Horseshoe Lake CA wetlands with elevated nitrate levels also contained high concentrations of ammonia, but wetlands as a whole were highly variable with respect to ammonia levels. Gordon (1989) observed that levels of nitrate and ammonia increased up to 5-fold in wetlands in the Rainwater Basin of Nebraska when large concentrations of waterfowl were present. Horseshoe Lake CA wetlands with elevated levels of both nitrate and ammonia (H3 and H4) periodically received high waterfowl use, but use had diminished during the 3 weeks prior to water sampling. Agricultural fields surrounding these wetlands may have been fertilized in early spring, thus accounting for elevated nitrogen levels.

Fall 1992.--Water chemistry profiles of wetlands and lakes sampled at Horseshoe Lake and Union County CA during October 1992 were similar to those sampled in March 1992. Ca, Mg, and SO_4 were again the principle dissolved ions present, and ph was near neutral for all wetlands (6.6-8.4). Concentrations of NO_3 and NH_3 were mostly below detection limits (Table 7). Differences in total conductivity and concentrations of Ca and Mg were similar to that observed in March 1992, with shallow wetlands (U2, U6, U8, U12) at

Table 7. Water quality measurements from wetland and lake sites on Horseshoe Lake and Union County Conservation Areas during October 1992.

Wetland Site	Temp. (°C)	Diss. O_2 (mg/l)	Conductivity (micromhos/cm^2)	pH	Na (mg/l)	Ca (mg/l)	Mg (mg/l)	Cl (mg/l)	SO_4 (mg/l)	NO_3 (mg/l)	NH_3 (mg/l)
Union County Conservation Area											
U1A	9.5	7.0	49	6.9	4.8	10.2	3.3	2	13	0.08	<0.5
U1B	8.0	10.6	53	8.4	5.4	11.3	3.8	4	10	<0.06	0.9
U2A	11.0	6.4	400	7.4	4.3	86.8	28.6	4	15	<0.06	<0.5
U2B	11.0	6.3	408	7.5	4.3	87.0	28.5	2	15	<0.06	<0.5
U6A	10.2	2.9	231	7.4	2.6	44.3	14.4	6	8	<0.06	<0.5
U6B	10.0	3.6	304	7.7	4.9	58.5	21.7	4	5	<0.06	<0.5
U8A	9.5	8.0	395	7.5	5.1	81.2	27.8	2	20	<0.06	<0.5
U8B	9.5	7.4	410	7.5	5.2	82.5	28.4	2	18	<0.06	<0.5
U12A	10.0	2.2	188	7.4	5.1	37.5	11.7	6	4	<0.06	<0.5
U12B	9.5	8.8	122	8.4	4.3	20.2	10.0	4	6	<0.06	<0.5
Horseshoe Lake Conservation Area											
H1A	11.5	3.6	86	7.6	3.5	16.9	4.0	2	5	<0.06	<0.5
H1B	11.0	2.2	118	7.1	3.8	19.9	5.5	4	7	<0.06	<0.5
H2A	11.0	8.0	106	7.3	<1.0	16.4	2.7	2	21	<0.06	<0.5
H2B	11.0	6.9	70	7.1	6.8	13.2	4.3	2	16	<0.06	0.7
H3A	10.0	6.8	49	6.9	1.3	4.6	2.3	2	5	<0.06	<0.5
H3B	11.0	4.5	90	6.8	1.0	5.1	2.6	2	8	<0.06	<0.5
H6A	12.0	6.2	78	7.4	3.9	14.0	4.3	4	5	<0.06	<0.5
H6B	12.0	6.3	75	7.3	4.1	14.1	4.4	2	5	<0.06	<0.5
H8A	11.5	6.4	41	6.6	1.5	6.4	2.2	2	9	0.14	<0.5
H8B	11.5	7.9	37	6.7	1.6	5.9	2.3	2	7	0.06	<0.5

Union County CA having the highest levels of these 3 parameters. The only lake sampled in October 1992 (U1) had characteristically low levels of conductivity, Ca, and Mg.

Shallow wetlands at Union County CA that were flooded by pumping from groundwater wells exhibited the highest conductivities and Ca and Mg concentrations during October 1992. Wetlands U2 and U8 contained little water prior to pumping and they had the highest levels of these 3 parameters (Table 7) because groundwater was not yet diluted by precipitation and surface water present before pump activation. Barker Lake (U6) contained water prior to pumping, and somewhat lower levels of Ca, Mg, and conductivity were observed because of dilution with surface water. All wetland and lake sites at Horseshoe Lake CA and wetlands and lake sites at Union County CA with natural water sources had lower levels of the Ca, Mg, and total conductivity than wetlands U2 and U8.

Nitrate and ammonia levels in water samples collected in October 1992 (Table 7) were lower than in March 1992 (Table 6). Detectable levels of ammonia and nitrate were observed in only 2 and 3 October 1992 samples, respectively, and these concentrations were minimal. During March 1992, 19 of 32 samples contained measurable nitrate, and 11 of 32 contained measurable ammonia. The higher levels of nitrate and ammonia detected in winter samples probably resulted from nitrogenous wastes that accumulated during winter when waterfowl were concentrated on these sites.

Prediction of Ca and Mg from conductivity.--Calcium concentrations in Union County and Horseshoe Lake CA wetlands and lakes were highly correlated ($\underline{r} = 0.9845$) with Mg concentrations, and both parameters were correlated with field conductivity measurements. Since concentrations of these cations were strongly and positively correlated, the inclusion of both in a multiple regression model explained changes in conductivity only slightly better than simple regressions containing Ca and Mg individually. A multiple linear regression model relating Ca and Mg concentrations to total conductivity explained 96.8% of variation in total conductivity. The amount of explained variation declined to 96.5% and 95.3%, respectively, in simple regression models using Ca and Mg concentrations as single independent variables.

The precision of single independent variable models for explaining cation-conductivity relationships allowed the use of field conductivity measurements to estimate Ca and Mg concentrations separately with relatively narrow 95% confidence limits. The least squares model for estimating Ca concentration from conductivity was:

$$\text{CALCIUM} = -2.011 + (0.224 * \text{CONDUCTIVITY})$$

where calcium is expressed as mg/l and conductivity is expressed as micromhos/cm. The 95% confidence interval for the predicted Ca level was ±10.4 mg/l.

The least squares model for estimating Mg concentration from conductivity was:

$$\text{MAGNESIUM} = -1.570 + (0.079 * \text{CONDUCTIVITY})$$

where magnesium is expressed as mg/l and conductivity is expressed as micromhos/cm. The 95% confidence interval for the predicted Mg level was ±4.2 mg/l.

These estimators were used to predict Ca and Mg concentrations from field conductivity measurements taken during bacteriological sampling in November and December 1992 (Table 8). Total conductivity and concentrations of Ca and Mg declined since October 1993 in wetlands at Union County CA that were filled from ground water wells (sites U2 and U6). Site U8 was not sampled in November or December 1992.

Table 8. _In situ_ measurements of selected water quality parameters taken from wetland and lake sites on Horseshe Lake and Union County Conservation areas during 16 November and 1 December 1992.

Wetland Site	Conductivity (micromhos/cm^2)		Dissolved O^2 (mg/l)		pH		Temperature (°C)		Predicted Ca^1 (mg/l)		Predicted Mg^1 (mg/l)	
	NOV	DEC	NOV	DEC	NOV	DEC	NOV	DEC	NOV	DEC	NOV	DEC
Union County												
U1	40	45	9.7	11.5	8.2	7.4	7.0	5.0	6.9	8.1	1.6	2.0
U2	392	345	7.4	10.5	7.6	7.5	11.0	5.5	85.8	75.3	29.4	25.7
U6	239	168	9.0	10.0	8.2	7.3	9.0	4.5	51.5	35.6	17.3	11.7
Horseshoe Lake												
H1	102	110	8.8	1.8	7.4	5.8	9.0	6.5	20.8	22.6	6.5	7.1
H2	51	53	11.5	8.5	8.2	7.4	11.0	6.0	9.4	9.9	2.5	2.6
H6	40	70	12.7	6.2	8.0	7.2	9.5	6.0	6.9	13.7	1.6	4.0

[1] Predictions of Ca and Mg concentrations were calculated from conductivity measurements as described in text.

Comparison with other enzootic sites.--If water quality chemistry is an important influence on persistence of P. multocida, then avian cholera enzootic sites should have characteristic physical and chemical profiles that enhance transmission of the disease agent among susceptible hosts, thereby producing more frequent and severe disease outbreaks. The ranges of conductivity (168-410 μmhos/cm^2), Ca (20-87 mg/l), and Mg (4-29 mg/l) levels in shallow wetland sites at Union County CA during March 1992 and October-December 1992 were higher than recorded on lake sites at Union County CA (conductivity, 40-67 μmhos/cm^2; Ca, 7-15 mg/l; Mg, 2-4 mg/l) and all sites at Horseshoe Lake CA (conductivity, 29-118 μmhos/cm^2; Ca, 2-23 mg/l; Mg, $<$1-7 mg/l). Higher conductivity, Ca, and Mg values in shallow wetlands at Union County CA coincided with more frequent and severe epornitics compared to Horseshoe Lake CA (see Job 1, Characteristics of Avian Cholera Outbreaks) and supports the notion that microenvironmental differences are associated with temporal and geographic patterns of avian cholera outbreaks (Friend 1981, Windingstad et al. 1988). However, based on comparisons with wetlands in the Rainwater Basin, shallow wetlands at Union County CA are not uniquely predisposed to disease outbreaks by the physical and chemical characteristics of surface water. Conductivity, Ca, and Mg in shallow wetlands at Union County CA were mostly below, or within the low range, of values reported for wetlands with histories of avian cholera in the Rainwater Basin (Windingstad et al.1988, Gordon 1989).

Culture and Isolation of P. multocida

Selective media tests.--Blood agar grew the most colony types and most of the Gram + organisms. However, luxuriant growth of Bacillus sp., Pseudomonas sp., and Proteus sp. on blood agar made it difficult to isolate other organisms. Rosen's medium inhibited growth of some organisms but also greatly inhibited growth of P. multocida (requiring 48 hrs for even modest growth), while S. typhimurium and E. coli grew well after 24 hours. Rosen's and the selective PM media inhibited growth of many Gram + organisms. Growth of Gram + bacteria was reduced on Rosen's medium, and even more on selective PM medium. PM selective media inhibited growth of most common organisms while still allowing P. multocida to grow almost as well as on blood agar. Spreading and swarming growth was mostly eliminated on PM selective media. Rosen's medium was not satisfactory because growth of P. multocida was inhibited and differentiation of colonies was difficult.

The 3 media were not compared quantitatively because not all colonies were selected for identification on all plates. However, PM selective medium plates grew fewer colony types, particularly in sediment samples that were difficult to read on blood agar because of overgrowth. Intensity of growth on PM selective media also was decreased, resulting in well isolated colonies. Thus, blood agar and Rosen's media that have been used by others to grow and isolate P. multocida from tissue samples were not suitable for growth and/or isolation of the bacterium from water or sediment samples. However, P. multocida was isolated from water and sediment samples inoculated with very low concentrations of the bacterium (4 organisms/ml) when grown in PM selective enrichment broth then plated on PM selective media.

Testing of the 4 different isolation procedures indicated that recovery of *P. multocida* inoculated into water samples collected from Barker Lake was best when samples were first incubated in PM selective enrichment broth and then streaked onto blood agar plates (Moore et al. 1994). *P. multocida* was recovered from 0% of control samples, and recovery rates increased as inoculated concentrations of *P. multocida* increased from 6 X 10^1 to 6 X 10^3 CFU/ml. Recovery rate was 84% at 6 X 10^1 CFU/ml and 100% at or above 6 X 10^2 CFU/ml. Recovery rates were lower when samples were first inoculated into PM selective enrichment broth and then plated on PM selective medium, with 32% recovery at 6 X 103 CFU/ml and 16% at 6 X 10^1 and 6 X 10^2 CFU/ml. Direct plating onto PM solid selective medium performed nearly as well as plating on blood agar after incubation in PM selective enrichment broth (Moore et al. 1994). Recovery was poorest with direct plating onto blood agar.

Environmental sampling.--We collected 350 surface water samples on 7 occasions during 22 October 1992 - 8 February 1993 at 3 wetland and lake sites each at Union County and Horseshoe Lake CA. Goose populations present at each Conservation Area during weeks that we sampled water were 3,000 - 56,000 birds at Union County CA, and 9,000 - 130,000 geese at Horseshoe Lake CA (Table 9). Despite the presence of as many as 8,000 (Union County CA) to 10,000 (Horseshoe Lake CA) geese using sample sites prior to collection, we detected no *P. multocida* in 700 subsample cultures of surface water (Table 10). Twenty surface water samples collected from Barker Lake at Union County CA on 27 October 1993 also were negative for *P. multocida*. Weather conditions that were associated with past outbreaks of avian cholera in southern Illinois (below normal temperatures,

Table 9. Total goose populations and numbers of geese recorded at sampling sites when water samples were collected for culture and isolation of Pasteurella multocida at Union County and Horseshoe Lake Conservation Areas (CA) during 22 October 1992 - 8 February 1993. See Figs. 1 and 2 for locations of sample sites.

	Union County CA				Horseshoe Lake CA			
Date	U1	U2	U6	Total[a]	H1	H2	H6	Total
10/22/92	-	-	-	3,500	-	-	-	9,000
11/16/92	-	-	-	3,000	-	-	-	9,000
12/01/92	500	2,000	100	25,000	400	800	2,000	38,000
12/16/92	8,000	600	7,000	56,000	1,000	2,000	5,000	75,000
01/11/93	3,000	500	200	45,000	1,000	0	5,000	130,000
01/25/93	5,000	600	1,000	40,000	500	0	5,000	85,000
02/08/93	100	0	1,000	19,000	5,000	10,000	1,000	58,000

[a] Weekly aerial survey estimates for total number of geese at each area.

Table 10. Results for culture and isolation of Pasteurella multocida (PM) from water samples collected on Horseshoe Lake and Union County Conservation Areas during 22 October 1992 to 7 February 1993.

Sampling Date	Number of samples tested	Number of subsamples from which PM like colonies were analyzed							Number of PM isolated
		PMSA[1]	BA[2]	GS[3]	OX[4]	TSI[5]	MAC[6]	API[7]	
10/22/92	50	100	40	16	16	16	0	1	0
11/16/92	50	100	44	44	33	33	2	2	0
12/01/92	50	100	33	33	21	21	21	2	0
12/16/92	50	100	29	29	17	0	0	17	0
01/11/93	50	100	32	32	22	22	22	4	0
01/25/93	50	100	50	0	0	0	0	0	0
02/08/93	50	100	50	3	3	3	3	3	0
Total	350	700	278	157	112	95	48	29	0

[1] PMSA = Pasteurella Multocida Selective Agar
[2] BA = Blood agar plates
[3] GS = Gram stains
[4] OX = Oxidase test
[5] TSI = Triple sugar iron agar
[6] MAC = MacConkey Agar
[7] API = API 20E identification system

extensive and prolonged snow and ice cover) occurred for the first time during this study in mid-January 1994. With 60,000 geese wintering at Union County CA and large numbers of geese using Barker Lake, we anticipated a possible disease outbreak. Six water samples collected from small areas of open water where high densities of geese were observed during cold weather were negative for presence of *P*. *multocida* and no outbreak occurred.

We failed to detect *P*. *multocida* in surface water despite the most intensive attempt yet to isolate the organism from the environment of a suspected enzootic site prior to the occurrence of a disease outbreak. Although unlikely, we cannot definitively conclude that water was not an environmental reservoir for the disease agent. *P*. *multocida* may have been be present in portions of wetlands that we did not sample, or it could have occurred in surface water at concentrations below detection limits. Based on testing of our culture and isolation procedures, we believe the organism would have been detected at some point in time if it was ubiquitously distributed within surface water of suspected enzootic sites at concentrations $\geq$ 6 X 10^1 organisms/ml. Also, the organism could have occurred elsewhere in the water column, in wetland substrate, or other elements of the physical or biological environments.

Job 3: Physiological Condition and Environmental Stresses

Objective: To evaluate relationships of physiological condition and environmental stresses to the occurrence of disease outbreaks and other sources of natural mortality.

INTRODUCTION

Whether or not an infection leads to disease in a host depends on properties of the infectious agent (e.g. a bacterium such as P. multocida) and also attributes of the host. Disease is the outcome when a host is unable to resist and/or overcome an infection. While it is clear that the precipitating cause of an avian cholera outbreak is successful transmission of a virulent strain of P. multocida from host-to-host, the predisposing environmental or host factors are less clear.

To better elucidate the relationships between physical condition, stress, and avian cholera outbreaks, we planned to (1) monitor goose habitat use and activity patterns, (2) determine movement patterns between roosting areas and rates of contact between birds using discrete roosting areas, (3) compare physiological condition between healthy and dead or moribund geese, and (4) monitor ambient conditions to identify periods of potential energetic or nutritional stress.

We monitored habitat use, activity, movements, and ambient conditions in the conduct of Job 1 (Characteristics of Avian Cholera Outbreaks). However, there was no evidence of mortality attributable to avian cholera (or other infectious diseases) so we altered the approach to this job. Briefly, we developed a protocol to establish baseline levels of hormonal indices in apparently healthy geese. The baseline data would have more general application to detect stress in geese and contribute to better understanding of the role of stress in the epizootiology of avian cholera.

METHODS

Geese were collected by live-trapping or shooting at Union County CA in 1993 and 1994. Geese captured in a swim-in trap were deeply anesthetized with an intramuscular injection of 20 mg/kg of ketamine hydrochloride and euthanized by cervical dislocation. Geese were sexed and aged (adult vs. juvenile) based on gonadal characteristics. All birds collected were in good flesh. No gross lesions were noted at necropsy so the birds were presumed to represent a "healthy" cohort.

Adrenals, livers, gonads, and thyroids were removed in 1993. The left of each paired organ (adrenal, testis, thyroid) was removed within minutes of death; the right organs were removed about 12 hours post mortem. Only adrenals were collected in 1994 and they were taken from birds necropsied within 4 hours of death. Collected tissues were chilled on wet ice until frozen for storage pending analyses.

The tissues were stored frozen (-20 C) until homogenized in a Tekmar Tissuemizer for 1 minute at 10,500 rpm in 5 ml of d_2H_2O. Homogenates were again frozen for storage until assayed for steroids.

Hormone concentrations were measured using solid-phase radioimmunoassay (RIA) kits with ^{125}I-tracers (Diagnostic Products Corp., Los Angeles, CA). Because these kits all use standard curves based on human serum, parallelism was determined between standard curves and the appropriate goose sample pools (Table 11). Data from RIAs were obtained using programs RIAPLOT and RIADOSE (Amador and Hodges 1989). Data were statistically analyzed using Student's t-test (Sokal and Rohlf 1981). Level of significance was set at $\underline{P} = 0.05$.

RESULTS

Body Organ Masses

Organs were collected from 22 geese during the period 16 January - 13 February 1993 (11 adult males, 6 adult females, and 5 juvenile females). Fourteen geese were collected during 27 January - 4 February 1994; 6 adult males, 4 adult females, 3 juvenile females, and 1 juvenile male). Body masses and measurements were not taken in 1993, but gross observations revealed the birds were in good physical condition. Mean fresh body masses were 4,212 g, 3,712 g, and 3,233 g for adult males, adult females, and juvenile females, respectively in 1994. The juvenile male weighed 3,907 g. All body masses were within the normal range expected for B. c. interior during late winter in southern Illinois (Gates 1989). Thus none of the birds collected evidenced nutritional stress based on body mass or gross appearance at necropsy.

Ovarian mass (675.2 ± 59.6 mg, $\underline{n}=6$) was greater $\underline{P} < 0.005$) than testicular mass (433.8 ± 41.1 mg, $\underline{n}=11$) in adult geese. Adrenal and hepatic masses did not differ ($\underline{P} > 0.05$) between sexes, or between adult and juvenile females (Table 12).

Table 11. Characterization of hormone radioimmunoassays for Canada geese samples. m=slope, Y^I=Y intercept, and r=correlation coefficient.

Hormone Sample	m	Y^I	r
Progesterone			
Canada goose adrenal homogenate	-1.632	0.220	-1.000
Canada goose testes homogenate	-1.632	0.216	-1.000
Canada goose ovary homogenate	-1.631	0.214	-1.000
Standard curve	-1.631	0.217	-0.996
Testosterone			
Canada goose adrenal homogenate	-1.674	0.415	-1.000
Canada goose testes homogenate	-1.669	0.412	-1.000
Canada goose ovary homogenate	-1.671	0.413	-1.000
Standard curve	-1.671	0.415	-1.000
Estradiol			
Canada goose testes homogenate	-1.961	4.345	-1.000
Canada goose ovary homogenate	-1.966	4.372	-1.000
Standard curve	-1.956	4.341	-1.000
Corticosterone			
Canada goose adrenal homogenate	-2.003	-4.285	-1.000
Standard curve	-2.001	-4.281	-0.999
Aldosterone			
Canada goose adrenal homogenate	-2.205	5.513	-1.000
Standard curve	-2.207	5.522	-0.997
Thyroxine			
Canada goose thyroid homogenate	-2.172	3.769	-1.000
Standard curve	-2.173	3.776	-0.999
Triiodothyronine			
Canada goose thyroid homogenate	-2.289	0.012	-1.000
Standard curve	-2.284	0.013	-0.997
Canada goose liver homogenate	-2.332	0.188	-1.000
Standard curve	-2.333	0.188	-0.998

Table 12. Weights (± SE [n]) of selected organs collected from Canada geese in southern Illinois, January-February 1993 and 1994.

Tissue	Ad M			Ad F			Juv F		
Gonadal (mg)	433.8	41.1	(11)	675.2	59.6	(5)	252.6	33.1	(5)
Adrenal (mg)	677.4	78.7	(17)	684.0	124.2	(10)	614.7	115.5	(7)
Hepatic (g)	98.9	11.3	(11)	74.8	11.6	(6)	83.2	14.3	(5)

Effects of Post Mortem Interval on Steroid Assays

Hormone assays of 1993 samples were not affected ($P > 0.05$ by post mortem interval (0 vs. 12 hrs). We concluded that samples from birds dead <12 hours would provide unbiased measures of tissue steroid levels. Therefore, birds collected in 1994 were taken to the laboratory for necropsy and removal of organs for steroid assays.

Tissue Hormone Concentration and Content

Adrenals.--Males had higher concentration ($P < 0.05$), but not content of adrenal progesterone and testosterone than females. Juvenile females had higher progesterone concentrations ($P < 0.025$) and aldosterone total content ($P < 0.05$) than adult females. Differences were not detected in other parameters measured (Tables 13 and 14).

Gonads.--Testis had higher concentrations of steroids than ovaries, except for estradiol which was higher in ovaries ($P < 0.01$). Juvenile females had higher ($P = 0.01$) concentrations of progesterone and testosterone than adult females, and both concentration and content of 17-hydroxyprogesterone were higher ($P = 0.01$) in juvenile versus adult female geese (Tables 13 and 14).

Thyroid and Liver Hormones.--We did not detect differences in levels of thyroid or liver hormones between sexes ($P > 0.05$). However, juvenile females had higher content of thyroid triiodothyronine that adult females ($P < 0.05$; Tables 13 and 14).

Table 13. Hormone concentrations (± SE) in tissues collected from Canada geese in southern Illinois, January-February 1993 and 1994.

Tissue/Hormone	Ad M		Ad F		Juv F	
Adrenal	n = 17		n = 10		n = 7	
progesterone (ng/mg)	1.57	0.34	0.89	0.15	2.15	0.51
corticosterone (ng/mg)	4.54	1.13	4.50	1.64	4.66	1.38
aldosterone (pg/g)	103.5	26.1	174.1	59.1	73.1	11.2
testosterone (pg/mg)	7.43	1.04	4.79	0.87	6.95	1.06
Gonads (pg/mg)	n = 11		n = 5		n = 5	
progesterone	48.97	13.16	13.95	6.38	225.90	150.30
17-OH-progesterone	24.38	8.01	1.75	0.66	20.66	6.49
testosterone	381.65	129.66	21.28	5.63	39.37	4.66
estradiol	1.33	0.36	10.94	3.63	19.87	6.58
Liver (pg/mg)	n = 11		n = 6		n = 5	
triiodothyronine	6.07	0.83	4.88	1.05	7.48	1.12

Table 14. Hormone content (± SE) in tissues collected from Canada geese in southern Illinois, January-February 1993 and 1994.

Tissue/Hormone	Ad M		Ad F		Juv F	
Adrenal (μg/adrenal)	n = 17		n = 10		n = 7	
progesterone	1.04	0.26	0.63	0.15	1.14	0.22
corticosterone	2.81	0.63	2.45	0.65	2.59	0.67
aldosterone (pg)	59.4	12.4	107.3	36.2	39.3	3.4
testosterone (ng)	4.50	0.64	3.06	0.65	4.55	1.27
Gonads (ng/gonad)	n = 11		n = 5		n = 5	
progesterone	20.71	5.68	9.09	4.31	52.29	33.60
17-OH-progesterone	111.12	4.35	1.12	0.44	47.65	12.50
testosterone	155.93	60.74	15.04	4.91	10.05	2.10
estradiol	0.54	0.14	6.69	1.78	4.28	0.96
Thyroid (μg/thyroid)	n = 9		n = 4		n = 3	
thyroxine	2.40	0.42	2.14	0.38	2.83	1.26
triiodothyronine (ng)	5.83	1.24	3.56	1.31	23.29	8.77
Liver (ng/liver)	n = 11		n = 6		n = 5	
triiodothyronine	628.1	121.1	382.9	112.3	650.2	189.8

Effects of Year on Corticosterone Levels

Adrenal corticosterone concentration did not differ ($P > 0.05$ between adult male and female geese so samples were grouped to test for differences between years. Concentrations of corticosterone in January-February 1993 samples (5.08 ± 1.19 ng/mg adrenal, $n = 16$) did not differ ($t = 0.77$, 23 df, $P > 0.05$) from concentrations in 9 samples collected January-February 1994 (3.55 ± 1.60 ng/mg adrenal).

DISCUSSION

Stress is the effect of physical, physiologic, or emotional factors that induce an alteration in an animal's homeostasis or adaptive state (Andrews 1993). While adverse effects of severe or prolonged stress on an animal's well being are known, stress occurs along a continuum and the onset is difficult to detect and measure. LeMaho et al. (1992) noted that the absence of stress cannot be deduced from only behavioral observations. We agree that this is clearly the case with Canada geese wintering in southern Illinois.

Alternatively, some investigators have examined mass and histological changes in organs and associated changes with stress (Mori and George 1978). Levels of blood corticoids and other hormones have been used by a number of researchers as a measure of stress (see LeMaho et al. 1992). However, much published data about levels of hormones in avian species reflect studies of serum (or plasma) hormones associated with reproductive events (Akesson and Raveling 1981, Bluhm et al. 1983), or molt and migration (Ketterson et al. 1991). We found no published data on hormone concentration or content in tissues of geese or closely related species. Therefore, the results of tissue hormone assays we presented in results best serve as baseline data, albeit based on small samples sizes.

We had no independent means of judging the degree of stress the birds we sampled were experiencing. However, the physical condition of the birds as evidenced by body mass and fat reserves suggested that nutritional stress was unlikely. The winter of 1992-93 was relatively mild so stress caused by ambient environmental conditions also was unlikely. Although the winter of 1993-94 was characterized by below freezing ambient temperatures during the collection period, corticosterone levels did not differ between years. We speculate that if stress were a factor during the collection period in 1994, the level was below a threshold that could be detected by our assays and sample sizes.

We demonstrated the feasibility of detecting and reliably measuring concentration and content of hormones in adrenals, gonads, liver, and thyroid of Canada geese that had been dead for periods up to 12 hours. By eliminating the need to sample serum of plasma (that must be obtained from living animals), it should be possible for researchers to evaluate stress in healthy, morbid, moribund, or even dead geese once adequate baseline data become available.

Job 4: Analysis and Report

Objective: To integrate published data and findings from this study to: 1) assess the potential for avian cholera to cause massive die-offs at Union County and Horseshoe Lake CA, 2) formulate management recommendations that will minimize mortality and spread of the disease during outbreaks, and 3) to address the potential to physically modify water areas to eradicate the disease from endemic areas or to minimize the impact on waterfowl of disease outbreaks.

INTRODUCTION

This job was completed through preparation of quarterly, annual and final project reports. Within Jobs 1-3 Jobs we have integrated the findings of this and previous studies to examine different epizootiological features of avian cholera outbreaks. A conceptual model of the epizootiology of avian cholera on southern Illinois waterfowl refuges is presented below. This conceptualization is necessary to understand the risks of waterfowl mortality due to avian cholera in southern Illinois. Under this job, we discuss some of the conclusions that may be drawn from this study, and present recommendations for future research and management alternatives to minimize the risk of substantial waterfowl mortality associated with future disease outbreaks.

EPIZOOTIOLOGY OF AVIAN CHOLERA IN SOUTHERN ILLINOIS

Based on this study, and thought provoking reviews by Friend (1981), Botzler (1991), and Wobeser (1992), we provide a conceptual model of the epizootiology of avian cholera in southern Illinois. In developing this model, we consider what is currently known of the etiological agent, its occurrence and distribution, source or reservoir, mode of transmission, and host susceptibility to avian cholera in wild waterfowl. The life cycle, pathogenicity, and and virulence of the etiological agent is generally well known (Rosen 1971). Occurrence and distribution is considered from the standpoint of spatial and temporal patterns of disease outbreaks in North America. Mode and route of transmission are also relatively well understood, but the most important and least understood issues are the source or reservoir mechanism, and factors that determine host susceptibility to avian cholera.

The regular occurrence of epornitics after the mid-1980's indicated that avian cholera had become an enzootic disease at Union County or Horseshoe Lake CAs. However, with the absence of epornitics in the past 4 winters, avian cholera can not be considered enzootic in southern Illinois. Despite intensive monitoring, we saw no evidence of the "smouldering" occurrence of disease involving small numbers of birds that has been observed elsewhere in the absence of more "explosive" epornitics (Botzler 1991, Wobeser 1992). Introduction of disease by infected or carrier birds may occur under specific weather conditions and migration patterns that cause contact between those birds and susceptible winter populations (Brand 1984, Friend 1987, Wobeser 1992). Thus, despite the absence of avian cholera from wintering grounds of MVP geese in the last 3 years, the past history of the disease suggests that it is almost certain to reappear in southern Illinois.

The probability of a disease outbreak occurring on a specific site is a complex function of a series of stochastic and ecological events that begins when previously infected or carrier birds arrive at a site. Outbreaks of avian cholera are more sporadic in the Mississippi Flyway compared to the more frequent, severe, and temporally and geographically consistent outbreaks that occur in the Pacific and Central Flyways. Spatial patterns of avian cholera epornitics suggest a recent shift from foci in southern Illinois where the disease first appeared in the Mississippi Flyway (Windingstad et al. 1983), to sites within the range of Eastern Prairie Population (EPP) Canada geese in Minnesota, Iowa, and Missouri. Avian cholera outbreaks have been more frequent and severe within the EPP range where there is greater mixing between snow and Canada geese, compared to epornitics within the range of Mississippi Valley Population (MVP) Canada geese where fewer snow geese are present during winter and migration periods (Bellrose 1980). Thus, outbreaks within the EPP range have typically involved mostly snow geese, whereas most outbreaks documented in the eastern portion of the Flyway have caused mortality primarily in Canada geese of the MVP. The spatial and temporal patterns of avian cholera epornitics in the Mississippi Flyway are consistent with suggestions of others (Brand 1984, Wobeser 1992) that snow geese infected on arctic breeding grounds may be important carriers of the disease.

In the absence of a disease outbreak, we did not obtain conclusive evidence to support or refute the environmental persistence or carrier bird hypotheses. However, the spatial and temporal patterns of disease outbreaks in the Mississippi Flyway (particularly southern Illinois), and results of water quality and bacteriological sampling indicate that the physical environment is not the source of new outbreaks. If the environment was indeed a reservoir for the disease agent, there should have been a more consistent temporal pattern of outbreaks. The onset of disease should be predictable from knowledge of weather conditions and behavior (i.e. crowding) of waterfowl associated with previous outbreaks. Although weather was generally mild during the 3 winters of this study, conditions (weather and crowding) that were conducive to epizootics in the 1980's finally developed in 1993-94, yet no outbreaks occurred. We agree with Backstrand and Botzler's (1986) conclusion that although water may be capable of transmitting P. multocida to susceptible animals during an outbreak, it is unlikely that water or soil are long-term environmental reservoirs of the organism. The validity of this conclusion would be enhanced if in future years we were able isolate P. multocida from the environment during, but not prior, to an epornitic of avian cholera.

If, as we believe, the environment is not a reservoir for the disease agent to persist in the absence of an outbreak, then a host population must be involved in maintaining the disease in the absence of regular outbreaks. P. multocida could be maintained in populations of snow and/or Canada geese by carrier birds, as normal flora that are opportunistically pathogenic, or exist in a latent state and causing disease only after host resistance is compromised by physiological stress. Although difficult, it is essential to a full understanding of epizootiology of avian cholera to determine how P. multocida might persist in host populations.

For disease transmission to occur, infectious birds must shed sufficient quantities of virulent P. multocida to constitute an infective dose for neighboring birds. Probability of transmission is determined by the frequency of contact between infectious and susceptible birds, and the accumulation of virulent bacteria in the environment. The probability that sufficient quantities of the bacterium accumulate in the environment is a function of: 1) density of infected, sick, or dead birds that contribute to accumulation of bacteria in surface water, 2) volume and flow of surface water that dilutes the disease organism, 3) physical and chemical properties of surface water that influence survival of the bacterium under ambient conditions and determine accumulation of the disease agent over short time-spans. Also important are factors that cause shedding of P. multocida by infected birds and that affect resistance of susceptible birds to infection and disease.

We believe that there are certain habitat features of disease foci that predispose them to regular disease outbreaks. These habitat features influence the potential for disease transmission primarily by 1) affecting densities and mixing of carrier, infected, and susceptible birds; and 2) through the build-up of infective doses that are a function primarily of surface water dilution, and secondarily the physical and chemical properties of surface water. Apparently low availability of lake and wetland habitats relative to the numbers of waterfowl that winter at Union County CA is the primary feature of southern Illinois wintering areas associated with patterns of past disease outbreaks. Although levels of water quality parameters thought to influence survival of P. multocida (conductivity, Ca, and Mg) were not high compared to enzootic sites in the Rainwater Basin, they were elevated in shallow wetlands at Union County CA compared to Horseshoe Lake CA where past disease outbreaks were less frequent and severe. Although certain areas (i.e. Union County CA) and wetland sites (i.e. Barker Lake) have physical and biological characteristics that predispose them to avian cholera outbreaks, they do not act as reservoirs for the disease agent. We believe that environmental characteristics of these areas place susceptible birds at greater risk of exposure to infection at Union County CA than at other wintering areas in southern Illinois. Similarly, environmental conditions associated with Barker Lake place susceptible birds at greater risk of exposure than at other wetland or lake sites at Union County CA.

Next, birds must be exposed to infective doses that accumulate in the environment. Probability of exposure is a function of bird densities on wetlands that are used for roosting at night and/or resting for extended periods during the day. Bird-to-bird contact is not a primary means of exposure because normal behavior of Canada geese does not result in frequent or prolonged direct contact between non-family or even family members (Raveling 1970; R. J. Gates, pers. observ.). As expressed by Wobeser (1992), disease will not spread in the population unless exposed birds become infected and develop disease. The probabilities of infection and onset of disease are probably the most complex and difficult to study components of any epidemiological model. The route, duration, and degree of exposure (infective dose) are important determinants of infection that can be feasibly measured. However, virulence of the disease agent, and resistance and immunological response of the host are probably the most important, but also most difficult to study determinants of infection and disease.

Environmental conditions operating in different ways at various spatial scales play an important role in epizootiology of avian cholera. First, inter-mixing of individuals from different species populations or sub-populations are probably directly or indirectly determined by continental or regional scale variations in weather patterns that alter migration routes or influence food availability, habitat quality, and/or population characteristics. Second, local weather conditions influence densities and residence times (measured in waterfowl use-days) of waterfowl on shallow wetland sites. Caithamer et al. (1992) determined that diurnal use of wetland and lake roost sites at Union County CA during winter by Canada geese was associated with cold weather conditions and snow and ice cover that also characterize periods when outbreaks of avian cholera have occurred (Windingstad et al. In Press). Ambient weather conditions at the water surface affect not only the physical and chemical environment of P. multocida that may be shed by infected or diseased birds, but also the energetic and physiological state of infected, carrier, and susceptible waterfowl. Although weather conditions associated with avian cholera outbreaks are not conducive to persistence of P. multocida in water, they may be offset by dense aggregations of birds on areas with limited availability of open water, and by inducing physiological or energetic stress that compromise host resistance to infection and disease.

IMPLICATIONS FOR RESEARCH AND MANAGEMENT

Information acquired from this and previous studies of the winter ecology of Canada geese on southern Illinois refuges provide a background of basic data needed to understand the epizootiology of avian cholera in southern Illinois. Techniques that were developed in part through this study provided tools that would be useful in future investigations of avian cholera outbreaks in southern Illinois. Selective microbiological media (Moore et al. 1994) tested in this study can be used to detect P. multocida in the environment and in waterfowl and other organisms before, during, and after future disease outbreaks. Baseline hormonal assays conducted in this study with healthy birds will be useful in determining the role of physiological or energetic stress in predisposing birds to infection and disease.

Intensive sampling of surface water at a potential outbreak site (Barker Lake) at Union County CA should be conducted in early fall each year to determine that P. multocida is not present at or above the detection threshold. Water sampling for bacterial isolation should be repeated as soon as possible after the first evidence of mortality, and continue through the entire course of a disease outbreak. Changes in physical and chemical parameters and ambient weather conditions need to be closely monitored during the course of an outbreak to determine how weather and other physical factors influence the onset, duration, and severity of disease outbreaks. Waterfowl use-days should be measured using nocturnal and diurnal observations conducted daily at outbreak sites. Intensive searches, recovery, and necropsy of dead and moribund birds should be conducted to monitor the duration and course of mortality during an outbreak. Concurrent collections of visibly healthy birds would provide adrenal tissues for hormonal radioimmunoassays to compare indices of physiological or energetic stress between infected and healthy birds. By relating changes in concentration and ubiquity of P. multocida isolated from surface water during an outbreak to daily changes mortality and bird densities, some understanding of minimum infective doses and critical bird densities necessary to initiate an outbreak under natural conditions may be gained.

Since the environment is likely not an important bacterial reservoir for avian cholera in southern Illinois, the disease cannot be "eradicated" in the sense of eliminating the disease agent. However, habitat manipulations can still be implemented to reduce the risk of disease, or minimize the duration and severity of future outbreaks. Friend (1981) and Wobeser (1992) at least partially attributed the recent spread of avian cholera outbreaks in North America to concentration of wintering and migrating waterfowl on progressively declining or deteriorating wetland habitats. Enhancing availability or promoting greater use of larger and deeper wetlands at Union County CA might reduce bird densities on wetland and lake sites where the risk of disease outbreaks is greatest. There are opportunities to expand the availability of shallow wetland habitats at Union County and Horseshoe Lake CA (SIUC Coop. Wildl. Res. Lab., unpubl.) that would benefit populations of waterfowl and other wetlands wildlife, and also help disperse dense aggregations of birds that increase risk of disease outbreaks.

The potential for avian cholera to cause massive mortality of a biologically significant proportion of MVP Canada geese is low. Most of the MVP is widely distributed among 5 separate refuge areas in southern Illinois and western Kentucky. Union County CA is the refuge that appears to be at greatest risk of disease outbreaks. Mortality at other locations appears to have resulted from infected birds moving from Union County CA to other refuges where risks of disease outbreaks are lower (i.e. Horseshoe Lake CA, Rend Lake and Ballard County WMAs). During the years of this study, 68,000 - 104,000 geese were at potential risk of exposure at Union County CA, compared to 130,000 - 205,000 geese at Horseshoe Lake CA. Considering maximum numbers of geese that we observed on individual wetland or lake sites that were foci of past disease outbreaks (i.e. Barker Lake), numbers of geese at immediate risk was 2,000-60,000 birds. Actual exposure rates would be impossible to measure, but the disease agent would have to be very ubiquitous for all geese present on an outbreak site to become exposed. All exposed geese will not necessarily develop infection and become sick, further reducing potential disease mortality. Because geese are widely distributed among several different wetland and lake sites on southern Illinois wintering areas, documented mortality during avian cholera outbreaks has been relatively light (9-6,000 geese).

Although only small portions of refuge populations are at immediate risk of exposure in any one area, the disease can spread if not contained at outbreak foci. Therefore, routine surveillance of wetland and lake sites that have been foci of past outbreaks should be conducted to detect onset of disease. Disease surveillance is generally conducted only after outbreaks are already evident and the opportunity to minimize risk of mortality and spread of disease is lessened. Regular surveillance of previous disease foci at Union County CA (i.e. Barker and Grassy Lakes) should be conducted during mid-December through mid-February when weather conditions are conducive to outbreaks of avian cholera. Such conditions are sub-freezing temperatures, snow, and ice cover that persist for several days and cause crowding of several thousand geese around small areas of open water during daylight hours. Intensive searches with spotting scope and by walking shorelines should be conducted to retrieve any dead or moribund waterfowl. Retrieved birds need to be examined by a qualified pathologist for evidence of infectious disease. Surveillance should be expanded to other sites at Union County and Horseshoe Lake CA when the first case of avian cholera is confirmed.

Current procedures already in place are probably adequate to minimize mortality in the event of an outbreak. Prompt retrieval and disposal of infected birds and carcasses is the most important action that can be taken to minimize mortality and spread of disease. Detailed records of daily mortality, wetland and lake sites where dead and moribund geese were collected, and age, sex, and species composition of carcasses recovered during outbreaks will provide important data needed to improve understanding of this disease. If possible, ground water can be pumped to dilute bacterial concentrations in surface water at outbreak sites. Hazing of geese is not likely to be effective and may cause spread of the disease to new areas.

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